# Report to the Senate Armed Services Committee and the House of Representatives National Security Committee

# on

# Department of Defense
# Animal Care and Use Programs 1995

# TABLE OF CONTENTS

# List of Figures

# List of Tables

# List of Acronyms

| | |
|---|---|
| AAALAC | Association for Assessment and Accreditation of Laboratory Animal Care International |
| AALAS | American Association of Laboratory Animal Science |
| ACLAM | American College of Laboratory Animal Medicine |
| APHIS | Animal and Plant Health Inspection Service |
| ASBREM | Armed Services Biomedical Research Evaluation and Management |
| AWA | Animal Welfare Act |
| AWIC | Animal Welfare Information Center |
| BRD | Biomedical Research Database |
| CRISP | Computer Retrieval Information of Scientific Projects |
| DDR&E | Director, Defense Research and Engineering |
| DoD | Department of Defense |
| DTIC | Defense Technical Information Center |
| ELISA | Enzyme Linked Immunosorbent Assay |
| FEDRIP | Federal Research in Progress |
| FY | Fiscal Year |
| IACUC | Institutional Animal Care and Use Committee |
| IG | Inspector General |
| ILAR | Institute of Laboratory Animal Resources |
| JDL | Joint Directors of Laboratories |
| JTCG | Joint Technology Coordinating Groups |
| LAM | Laboratory Animal Medicine |
| MATRIS | Manpower and Training Research Information Services |
| NIEHS | National Institute of Environmental Health Sciences |
| NIH | National Institutes of Health |
| NTP | National Toxicology Program |
| OPRR | Office for the Protection from Research Risks |
| OSD | Office of the Secretary of Defense |
| PADPRP | Poly (ADP-ribose) Polymerase |
| PCR | Polymerase Chain Reaction |
| PHS | Public Health Service |
| RDT&E | Research, Development, Test, and Evaluation |
| S&T | Science and Technology |
| SEB | Staphylococcus Enterotoxin B |
| STO | Science and Technology Objective |
| TAPSTEM | Training and Personnel Systems Science and Technology Evaluation and Management |
| USAMRMC | United States Army Medical Research and Materiel Command |
| USDA | United States Department of Agriculture |
| WRAIR | Walter Reed Army Institute of Research |

# SECTION V
# DoD ANIMAL USE BY RESEARCH CATEGORY

The information presented in this section provides profiles on the use of animals in various research categories, and the U.S. Department of Agriculture (USDA) pain categories of Department of Defense (DoD) animal-based research, testing and training programs for fiscal year (FY) 1995.

## V.1 METHODS

Information was solicited and received from DoD agencies and military commands, organizations, and activities involved in animal care and use programs located both inside and outside of the United States. This included extramural contractors and grantees that performed animal-based research. For the purpose of this reporting requirement, an intramural program represents research performed at a DoD facility and funded by either DoD or non-DoD funds. An extramural program represents research performed by a contractor or grantee that is funded by the DoD.

### V.1.1 Animal Use Profiles

The animal use profiles prepared for this report are consistent with the reporting information and data provided to the USDA using the Animal and Plant Health Inspection Service (APHIS) Form 7023. In addition, this report contains comprehensive information on all other animals (i.e., mice, rats, birds) used that are not required in reports to the USDA.

For the purposes of this reporting requirement, an animal was defined as any whole nonhuman vertebrate, living or dead, excluding embryos, that was used for research, development, test, and evaluation (RDT&E), clinical investigations, diagnostic procedures, and/or instructional programs. Only live animals or whole dead animals, as defined, that were either on hand in the facility or acquired during FY95 were included. Animal organs, tissues, cells, blood, fluid components, and/or by-products purchased or acquired as such animal/biological components are not reported. This definition does not include animals used or intended for use as food for consumption by humans or animals, animals used for ceremonial purposes, or military working animals and their training programs.

A single animal was counted only once in determining the number of animals used during the fiscal year for a particular work unit or protocol. This does not refer to the number of times an individual animal is injected, manipulated, handled, or administered medication and/or experimental compounds within a given work unit, protocol, or program. Animals on hand during FY95, but not actually used during the fiscal year, are not included in this number.

### V.1.2 Animal Use Categories

All DoD agencies and military commands, organizations, and activities involved in the performance and/or funding of animal care and use programs reported animal work by the category that best describes the general purpose of the animal use. If these categories did not describe the animal use within a particular work effort, the animal was placed under the Other category. The 8 general categories and 23 specific subcategories are listed in Table V-1. In-depth information on specific activities performed within a subcategory is presented in Appendix K. The medical research categories correspond to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee's Joint Technology Coordinating Group Medical Research Areas. Non-medical categories consist of RDT&E programs performed outside the ASBREM Committee medical oversight. Clinical Investigations studies were performed under the auspices of the Assistant Secretary of Defense for Health Affairs and the military services medical departments through Major Force Program 8 funding. These studies were usually in support of graduate medical education training programs located at the major military medical centers.

*Table V-1 Animal Use Categories*

**MEDICAL (M)**
- M1: Military Dentistry
- M2: Infectious Diseases
- M3: Medical Chemical Defense
- M4: Medical Biological Defense
- M5: Human Systems Technology
- M6: Combat Casualty Care
- M7: Ionizing Radiation
- M8: Other Medical RDT&E

**NON-MEDICAL (N)**
- N1: Physical Protection
- N2: Physical Detection
- N3: Offensive Weapons Testing
- N4: Other Non-Medical RDT&E

**CLINICAL INVESTIGATIONS (C)**
- C1: Clinical Medicine
- C2: Clinical Surgery
- C3: Other Clinical Investigations

**TRAINING/INSTRUCTIONAL (T)**
- T1: Training, Education, and/or Instruction for Personnel
- T2: Other Training/Instruction

**ADJUNCTS/ALTERNATIVES TO ANIMAL STUDIES (A)**
- A1: Adjuncts to Animal Use Research
- A2: Alternatives to Animal Investigation
- A3: Other Alternatives/Adjuncts

**CLASSIFIED SECRET OR ABOVE STUDIES (S):** Classified secret or above studies on animals

**ANIMAL BREEDING STOCK (B):** Animals maintained for breeding

**OTHER ANIMAL USE CATEGORIES (O):** Other animal use purposes

## V.1.3 USDA Pain Categories

The USDA requires that all institutions using any regulated animal for research, testing, training, or experimentation register with the USDA as a research facility and submit an annual report. This annual report presents the number of regulated animals used and the type of pain, if any, the animals were exposed to.

The USDA has developed three pain categories for its reporting requirement (TableV-2). All animals herein reported are assigned to one of the three USDA pain categories; this includes animals that are not regulated by the USDA. The USDA requires that any reporting facility that uses procedures producing unalleviated pain or distress file an explanation of the procedures with its annual APHIS report.

The animals reported in Column C of the USDA report are those used in procedures that are not painful. Procedures performed on these animals are those that are usually conducted on humans without anesthesia or analgesia. Examples include most blood sampling techniques (excluding intracardiac and periorbital blood sampling), injections and tattooing.

The animals reported in Column D of the USDA report are those that experience pain in which appropriate anesthetics, analgesic or tranquilizing drugs were used. Examples include anesthesia for surgical procedures or catheter placement, and analgesia during recovery from surgery.

The animals reported in Column E of the USDA report are those that experience more than slight or momentary pain or distress that cannot be alleviated by drugs. Examples of procedures where drugs were not used because they would have adversely affected the procedures, results or interpretation of the research, or tests include some infectious disease studies and some toxicology studies.

All procedures that involve animals in Columns D or E are extensively reviewed during the protocol approval process. A veterinarian with experience and/or training in laboratory animal medicine must review all procedures that could cause pain and distress in animals. In addition, the primary investigator must write a justification for all procedures for animals in Columns D and E.

*Table V-2 USDA Pain Categories (USDA APHIS form 7023)*

**USDA COLUMN C**
Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.

**USDA COLUMN D**
Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.

**USDA COLUMN E**
Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

The DoD standard protocol states, "Procedures causing more than transient or slight pain that are unalleviated, must be justified on a scientific basis in writing by the primary investigator. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized." Moreover, the primary investigator must sign an assurance statement that alternative procedures are not available, and the Institutional Animal Care and Use Committee must review and approve all procedures before the study begins.

## V.2 Results/Discussion

### V.2.1 General Results

There was a total of 431,879 animals used in FY95 which is a 28% decrease from FY94 and a 22% decrease from FY93 (Figure V-1). The Animal Welfare Act of 1985 defines animals as "any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warmblooded animal, as the Secretary may determine..." Therefore, only 7% (28,245) of the animals used by the DoD in FY95 are considered USDA reportable species.

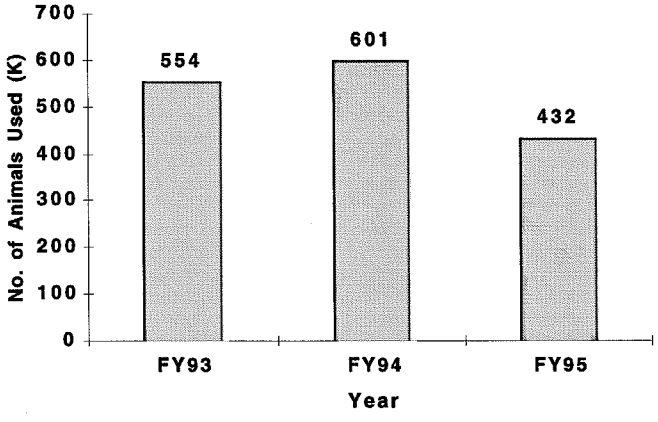



*Figure V-1 DoD Animal Use by Year*

In FY95 228,525 animals were used in intramural research programs and 203,354 were used in extramural grants or contracts (Figure V-2). There was a 15% and 39% decrease in FY95 intramural and extramural animal use, respectively. The decreased use of animals by extramural programs accounts for 77% of the total FY95 decrease. By their very nature, extramural research programs have the greatest fluctuation in the

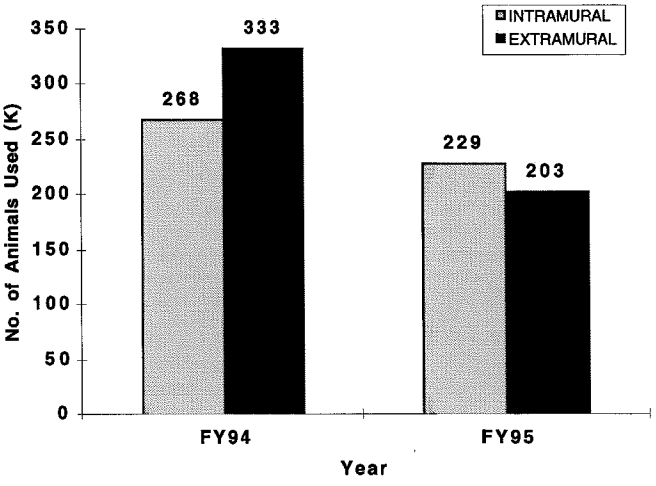



*Figure V-2 Intramural/Extramural Animal Use by Year*

number of animals used from year to year. Each year a different number of contracts are granted to perform extramural research. Many of these do not use animals at all; others only use animals during a portion of the proposed project (i.e., third year of project); and others use animals throughout the entire project. In addition, the level of funding for extramural programs varies from year to year thereby changing the total number of extramural projects. Some extramural research programs are congressionally mandated such as the Breast Cancer Research Program in which funding is dependent on yearly congressional appropriations. Therefore, changes in the number of animals used by the DoD extramural research programs can fluctuate significantly from year to year. The intramural programs have less variation in their use of animals because they have a continuous mission and ongoing research in specific areas. Consequently, any decrease in the number of animals used is most likely a result of the use of alternatives to animal use or decrease in the number of research projects.

### V.2.2 Animal Use by Service

Information concerning total DoD use of animals by each service is presented in Figure V-3. Figures V-4 and V-5 show the intramural and extramural animal use by service, respectively.

In FY95, the Army used 74% of the DoD total animal use, 58% of the total intramural animals and 92% of total extramural animals.

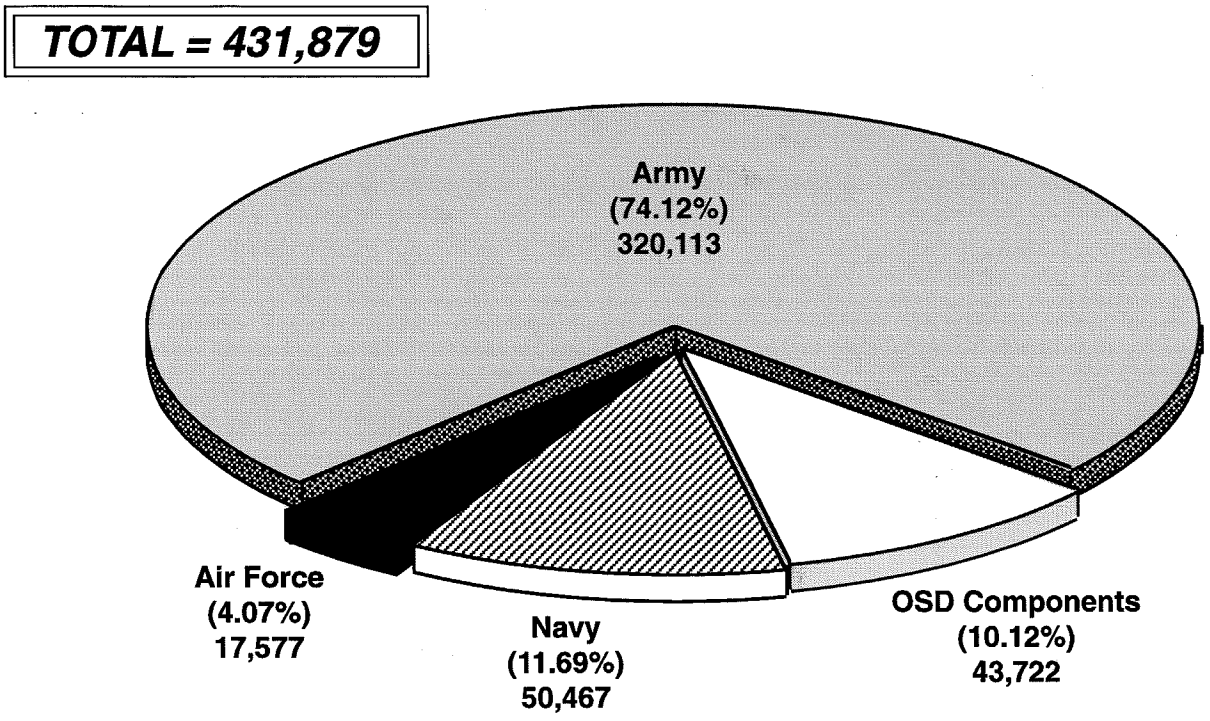



*Figure V-3 Total DoD Intramural and Extramural Animal Use by Service for FY95*

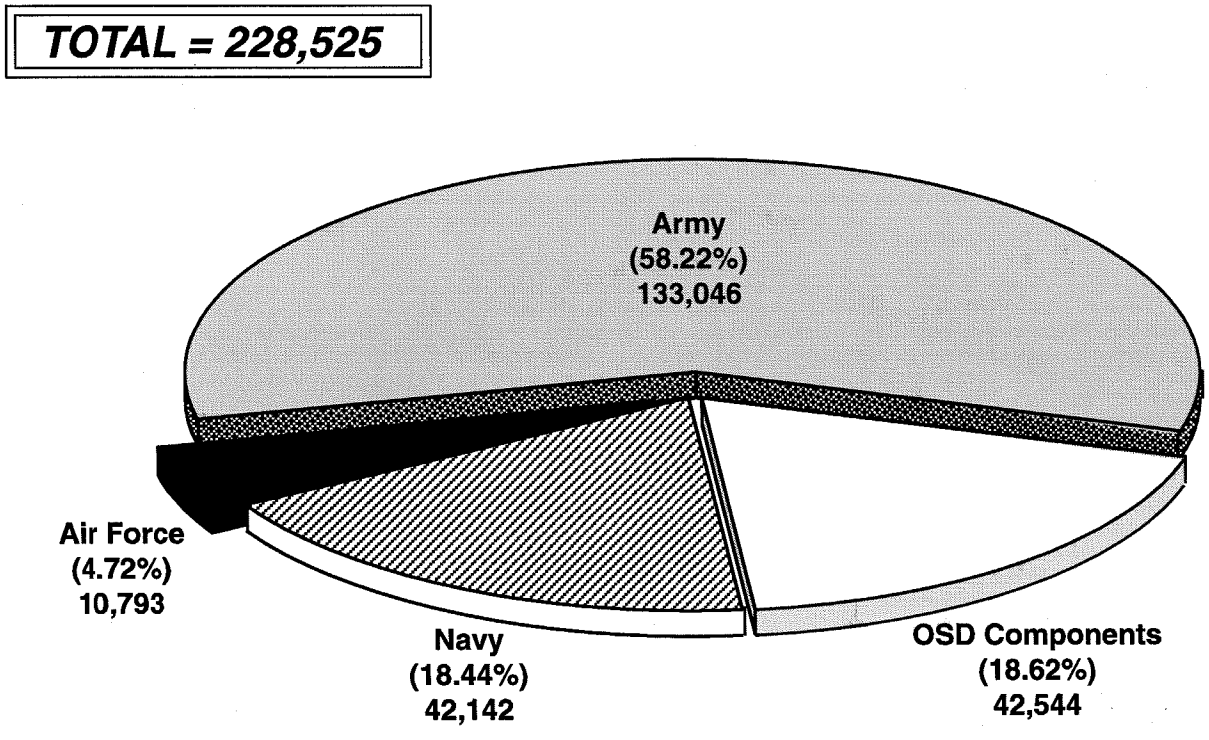



*Figure V-4 Total DoD Intramural Animal Use by Service for FY95*

**Percentages may not add up to 100% due to rounding of calculations**

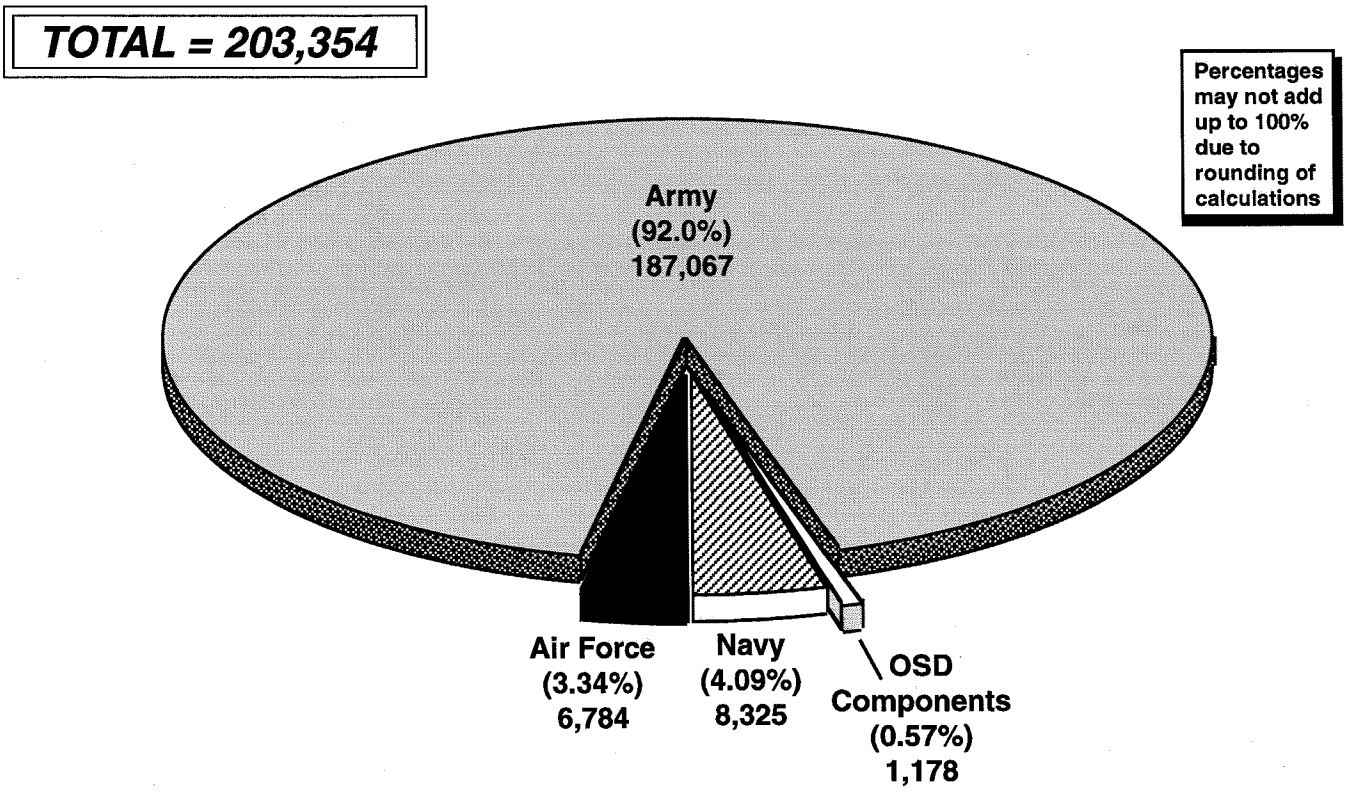



***Figure V-5*** *Total DoD Extramural Animal Use by Service for FY95*

The U.S. Army Medical Research and Materiel Command is the congressionally mandated Lead Agency for infectious disease and combat dentistry research and the DoD Executive Agent for medical chemical and medical biological defense and nutrition studies. The Command is responsible for greater than 85% of the DoD Medical Research, Development, Test and Evaluation programs. In addition, the Army has an ongoing responsibility to manage the congressionally mandated Breast Cancer Research Program. The Army had a 30% decrease in the number of animals used in FY95 resulting from a 105,196 decrease (36%) in the number of animals used in the Army's extramural research programs.

The Navy used 12% of the DoD total animal use, 18% of the total intramural animals and 4% of total extramural animals. In FY95, the Navy used 5,666 more animals, with the majority of these animals used in intramural research programs. There was a slight decrease in the Navy's extramural animal use for FY95.

The Air Force used 4% of the DoD total animal use, 5% of the total intramural animals and 3% of total extramural animals. The Air Force decreased the number of animals used in research by 20,401 animals (54%) in FY95. Most of this decrease (18,852) was in the Air Force's extramural research projects.

The Office of the Secretary of Defense (OSD) components are the Uniformed Services University of the Health Sciences, Advanced Research Projects Agency, Armed Forces Radiobiology Research Institute, and Armed Forces Institute of Pathology. OSD components used 10% of the DoD total animal use, 18% of the total intramural animals and less than 1% of total extramural animals. There was a 26% (15,259) decrease in the use of animals for the OSD components in FY95.

## V.2.3 Animal Use by Species

DoD animal use by species is presented in Figure V-6. Figures V-7 and V-8 represent the intramural and extramural animal use by species for FY95. The majority (~97%) of animals used by the DoD, both intramurally and extramurally, were rodents, birds, amphibians and fish. The numbers of both nonhuman primates and dogs and cats decreased in FY95 (Figure V-9).

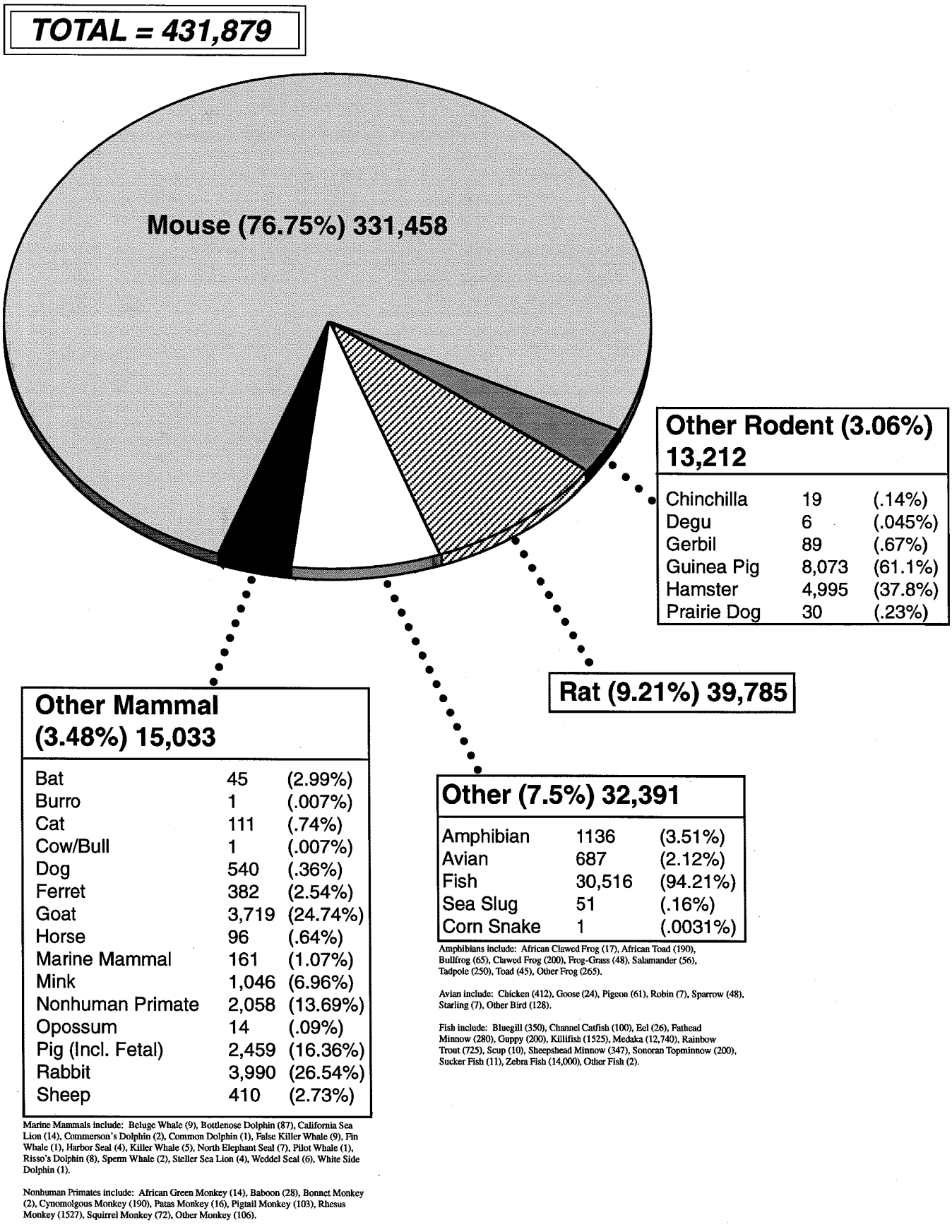
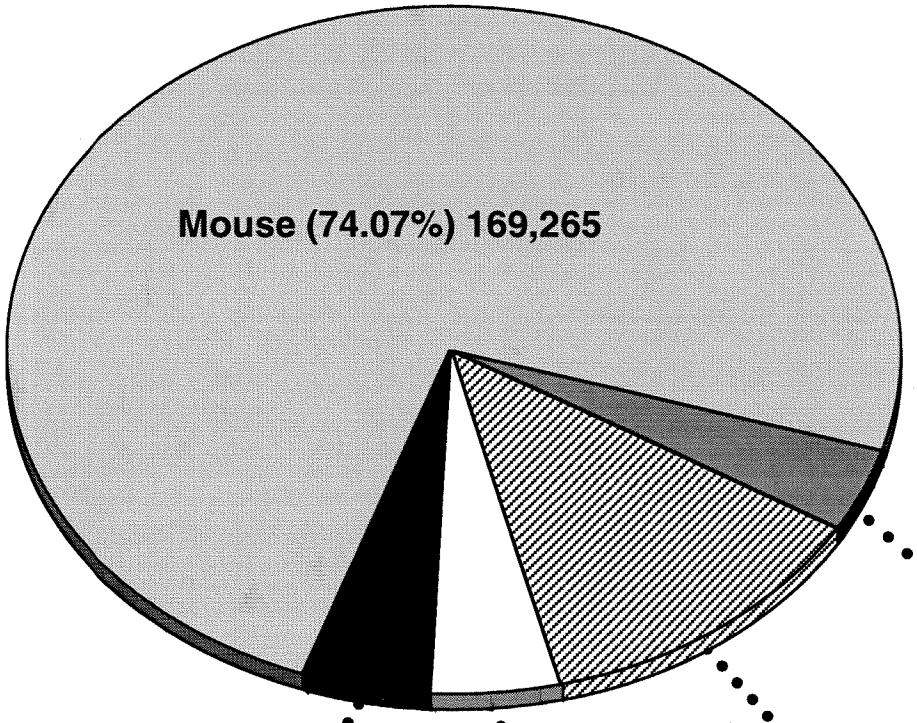



***Figure V-6*** *Total DoD Intramural and Extramural Animal Use by Species for FY95*

**TOTAL = 228,525**




**Other Rodent (3.9%) 8,916**

| | | |
|---|---|---|
| Chinchilla | 19 | (.213%) |
| Gerbil | 37 | (.41%) |
| Guinea Pig | 5,105 | (57.26%) |
| Hamster | 3,725 | (41.78%) |
| Prairie Dog | 30 | (.34%) |

**Rat (12.67%) 28,945**

**Other Mammal (4.82%) 11,007**

| | | |
|---|---|---|
| Burro | 1 | (.009%) |
| Cat | 90 | (.82%) |
| Cow/Bull | 1 | (.009%) |
| Dog | 340 | (3.09%) |
| Ferret | 267 | (2.43%) |
| Goat | 3,683 | (33.46%) |
| Horse | 77 | (.70%) |
| Marine Mammal | 44 | (.40%) |
| Nonhuman Primate | 1,458 | (13.24%) |
| Opossum | 14 | (.013%) |
| Pig (Incl. Fetal) | 2,144 | (19.48%) |
| Rabbit | 2,592 | (23.55%) |
| Sheep | 296 | (2.69%) |

Marine Mammals include: Beluge Whale (3), Bottlenose Dolphin (33), California Sea Lion (5), False Killer Whale (1), Risso's Dolphin (2).

Nonhuman Primates include: African Green Monkey (14), Baboon (5), Bonnet Monkey (2), Cynomolgous Monkey (165), Patas Monkey (16), Rhesus Monkey (1,104), Squirrel Monkey (51), Other Monkey (101).

**Other (4.54%) 10,392**

| | | |
|---|---|---|
| Amphibian | 664 | (6.39%) |
| Avian | 268 | (2.58%) |
| Fish | 9,408 | (90.53%) |
| Sea Slug | 51 | (.49%) |
| Corn Snake | 1 | (.0096%) |

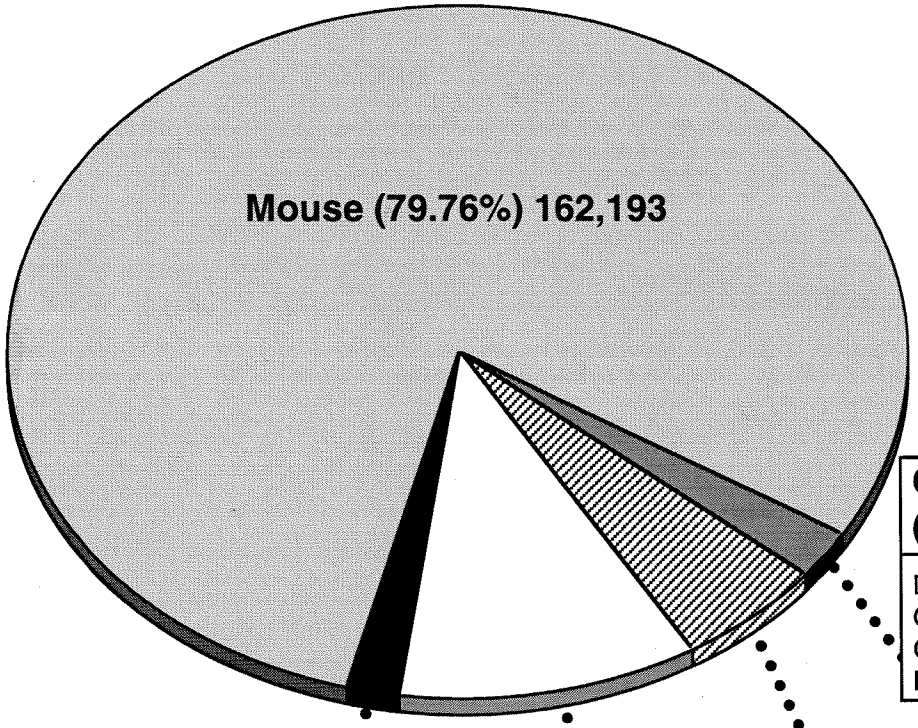
Amphibians include: African Clawed Frog (17), African Toad (190), Bullfrog (60), Clawed Frog (100), Frog-Grass (48), Salamander (56), Other Frog (193).

Avian include: Chicken (41), Goose (24), Pigeon (61), Robin (7), Starling (7), Other Bird (128).

Fish include: Bluegill (350), Guppy (200), Killifish (1500), Medaka (7,000), Sheepshead Minnow (347), Sucker Fish (11).

***Figure V-7** Total DoD Intramural Animal Use by Species for FY95*

**TOTAL = 203,354**




**Other Rodent (2.11%) 4,296**

| | | |
|---|---|---|
| Degu | 6 | (.14%) |
| Gerbil | 52 | (1.21%) |
| Guinea Pig | 2,968 | (69.09%) |
| Hamster | 1,270 | (29.56%) |

**Rat (5.33%) 10,840**

**Other Mammal (1.98%) 4,026**

| | | |
|---|---|---|
| Bat | 45 | (1.12%) |
| Cat | 21 | (.52%) |
| Dog | 200 | (4.97%) |
| Ferret | 115 | (2.86%) |
| Goat | 36 | (.89%) |
| Horse | 19 | (.47%) |
| Marine Mammal | 117 | (2.91%) |
| Mink | 1,043 | (25.91%) |
| Nonhuman Primate | 600 | (14.9%) |
| Pig (Incl. Fetal) | 315 | (7.82%) |
| Rabbit | 1,398 | (34.72%) |
| Sheep | 114 | (2.83%) |

Marine Mammals include: Beluge Whale (6), Bottlenose Dolphin (54), California Sea Lion (9), Commerson's Dolphin (2), Common Dolphin (1), False Killer Whale (8), Fin Whale (1), Harbor Seal (4), Killer Whale (5), North Elephant Seal (7), Pilot Whale (1), Risso's Dolphin (6), Sperm Whale (2), Steller Sea Lion (4), Weddel Seal (6), White Side Dolphin (1).

Nonhuman Primates include: Baboon (23), Cynomolgous Monkey (25), Pigtail Monkey (103), Rhesus Monkey (423), Squirrel Monkey (21), Other Monkey (5).

**Other (10.82%) 21,999**

| | | |
|---|---|---|
| Amphibian | 472 | (2.15% |
| Avian | 419 | (1.9% |
| Fish | 21,108 | (95.95%) |

Amphibians include: Bullfrog (5), Clawed Frog (100), Tadpole (250), Toad (45), Other Frog (72).

Avian include: Chicken (371), Sparrow (48).

Fish include: Channel Catfish (100), Eel (26), Fathead Minnow (280), Killifish (25), Medaka (5,740), Rainbow Trout (725), Scup (10), Sonoran Topminnow (200), Zebra Fish (14,000), Other Fish (2).

***Figure V-8** Total DoD Extramural Animal Use by Species for FY95*

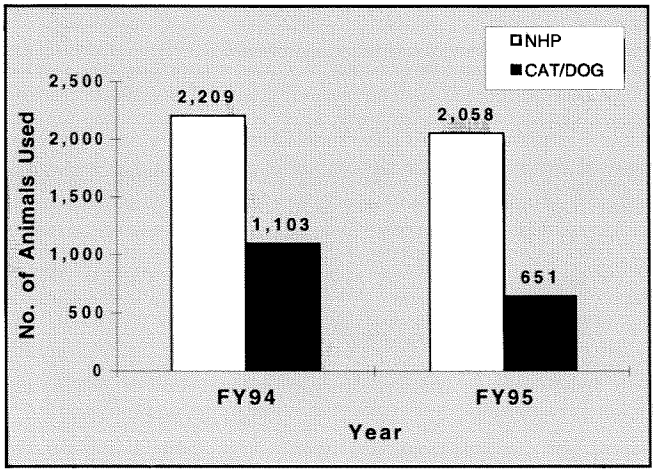
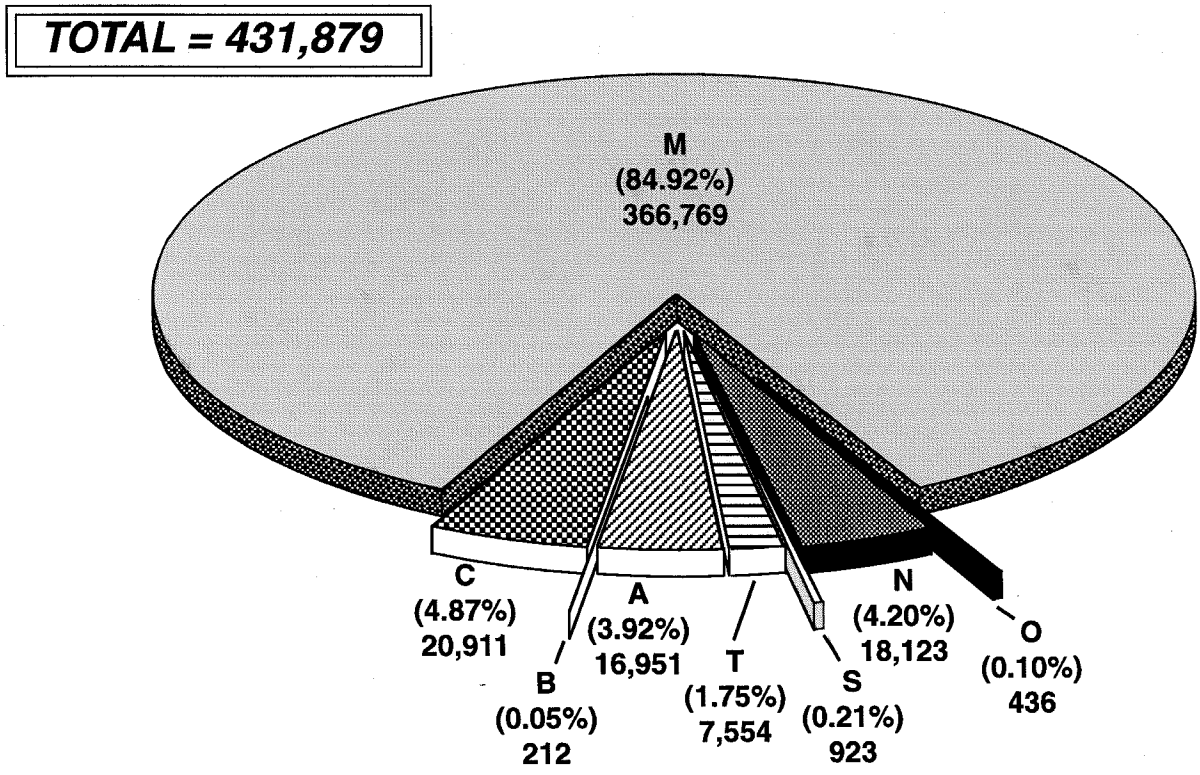



***Figure V-9*** *Use of Nonhuman Primates, Dogs, and Cats by Year*

## V.2.4 Animal Use by Category

Total animal use in the DoD by category is presented in Figure V-10, with the intramural and extramural breakouts in Figures V-11 and V-12, respectively. The DoD has a critical and challenging mission: to discover, design and develop military medical countermeasures against threats to the health and survivability of military personnel. In order to meet this mission, 90% of the animals used by the DoD in FY95 were in medical and clinical research. The majority (63%) of animals used in medical research were in the area of infectious diseases and were primarily rodents (99%) (Appendix L). The primary thrust of this research is the development of preventive measures against infectious disease through discovery, design, and development of prophylactic, therapeutic, and treatment drugs for relevant diseases. Ninety-one percent of the animals used in clinical research were used in clinical medicine studies.




A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

***Figure V-10*** *Total DoD Intramural and Extramural Animal Use by Category for FY95*

**TOTAL = 228,525**

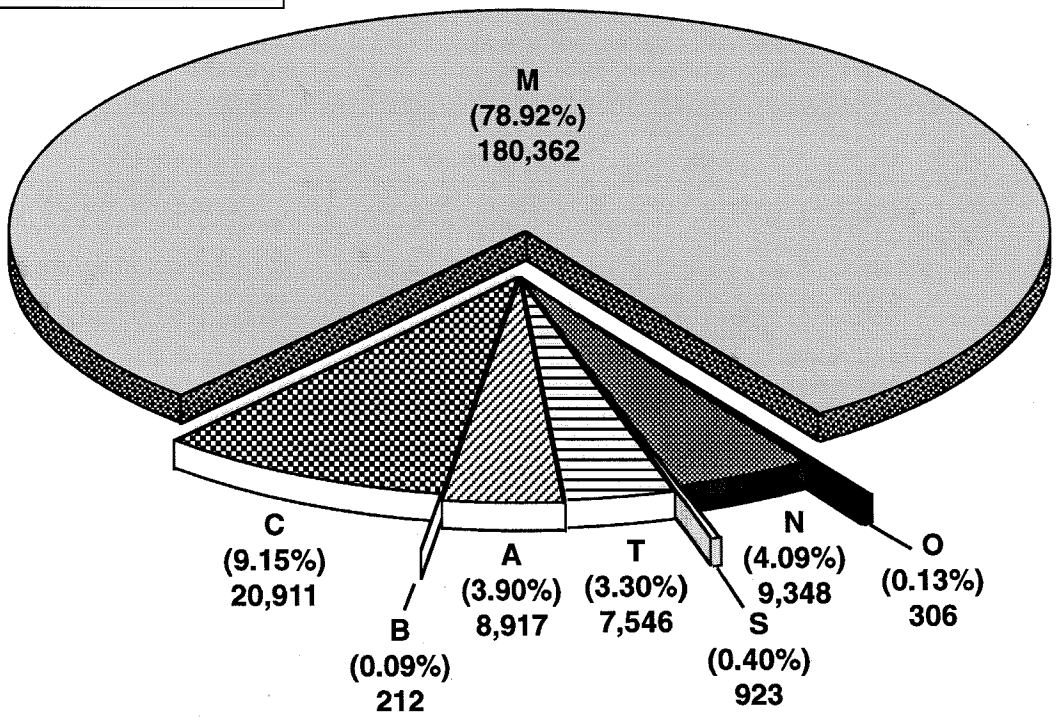



***Figure V-11*** *Total DoD Intramural Animal Use by Category for FY95*

**TOTAL = 203,354**

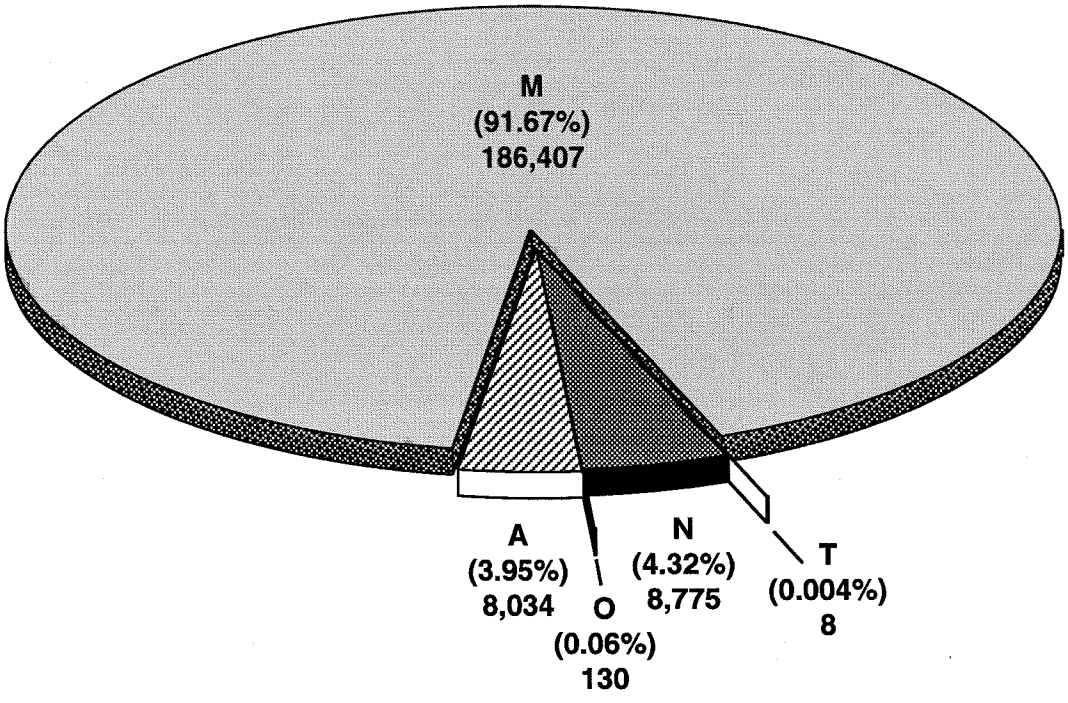



***Figure V-12*** *Total DoD Extramural Animal Use by Category for FY95*

A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

Non-medical RDT&E animal use accounted for only 4% of the total animal use in FY95. The use of animals in non-medical research has steadily declined during the past 3 years. Research in the area of alternatives to the use of animals was 4% of the total animal use for FY95 and utilized primarily fish (98%). Research in this category illustrates the Department's continuing initiatives to promote research to develop alternatives to reduce, replace and refine the use of animals in DoD research. In addition to the adjunct protocols focusing specifically on animal husbandry and care, in FY95 there are several ongoing actions in this area. As an example, Walter Reed Army Institute of Research has established a policy (WRAIR Policy Letter 93-27, Appendix M) that mandates consideration for environmental enrichment for research animals. This policy allows for flexibility and creativity for improving conditions of laboratory animals. No animals were used for offensive weapons testing during FY95.

## V.2.5 Animal Use by USDA Pain Category

Total animal use in the DoD by USDA pain category is presented in Figure V–13, with the intramural and extramural breakouts in Figures V-14 and V-15, respectively. Most research (~78%) in the DoD was not painful to the animals involved. In the majority of the cases (62%), the animals were not exposed to or involved in any painful procedures. In 16% of the cases, animals were given anesthesia or pain-relieving drugs during procedures that could have involved some pain or distress to the animals. In 22% of the animals used, anesthetics or analgesics were not used because they would have interfered with the results of experiments. Most (99%) of the animals used in painful experiments (where the drugs would have interfered with the results) were rats and mice. These rodents were used in medical, non-medical, and clinical research studies. There were no animals

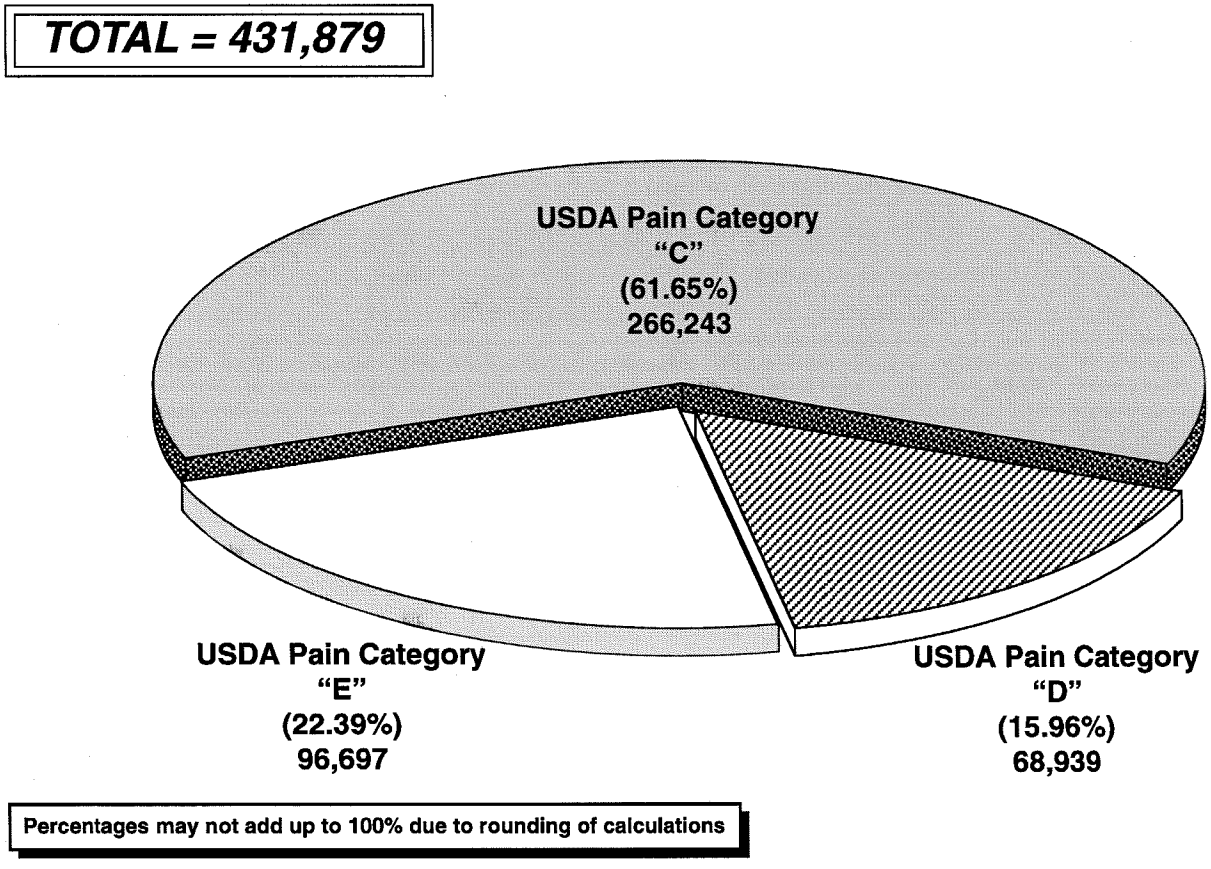



***Figure V-13*** *Total DoD Intramural and Extramural Animal Use by USDA Pain Category for FY95*

**TOTAL = 228,525**

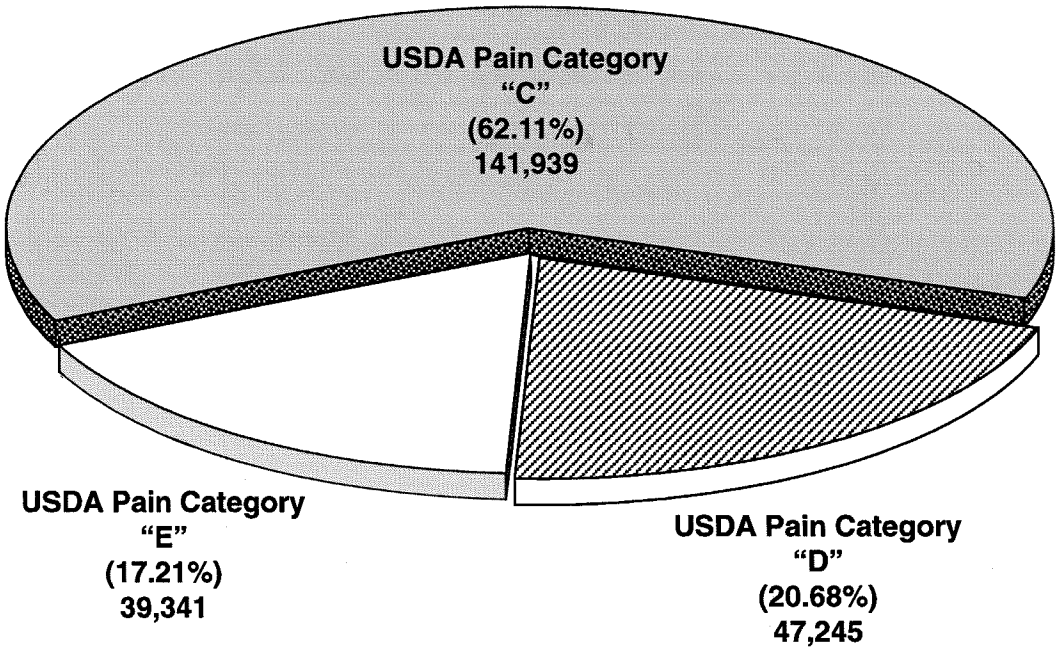



*Figure V-14 Total DoD Intramural Animal Use by USDA Pain Category for FY95*

**TOTAL = 203,354**

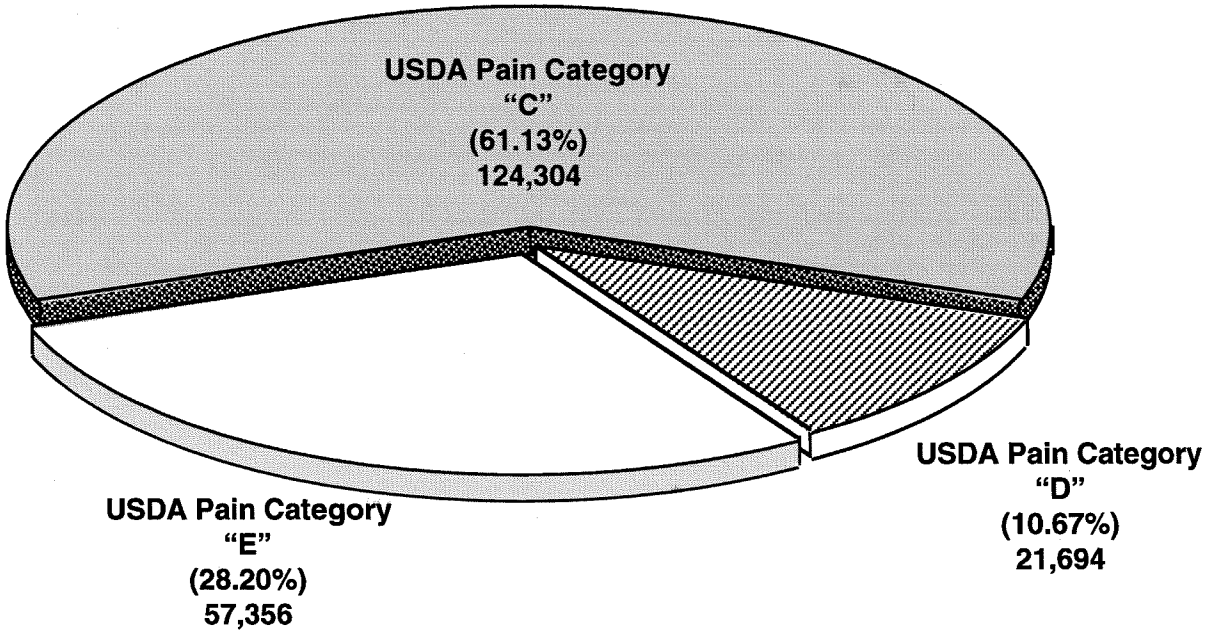



*Figure V-15 Total DoD Extramural Animal Use by USDA Pain Category for FY95*

**Percentages may not add up to 100% due to rounding of calculations**

subjected to unalleviated pain during training, secret, or alternative research studies. The DoD clearly has the most diverse, unique, and demanding R&D mission. The modern battlefield is a hostile and dangerous environment with extraordinary potential for exposure to lethal or debilitating conventional weapons, exotic endemic diseases, biological and chemical agents, nuclear blast and radiation, directed energy sources, and complex and dangerous equipment. In addition, a host of adverse environmental conditions, such as cold, heat, high and low pressure, are of grave concern. The DoD must provide acceptable protection against these threats and many others. The animals reported in Category E were used in the study of militarily relevant infectious disease, biowarfare defense, or chemical warfare defense efforts. This is critical research whose success is often reliant upon animal models for vaccine and efficacious counter-measure development. A large portion of these studies is driven by federal requirements, particularly those of the Food and Drug Administration. Research of this kind is not commonly done elsewhere in the government, academic, or private sectors.

# SECTION VI

# DoD INITIATIVES TO PROMOTE ALTERNATIVE METHODS THAT REPLACE, REDUCE AND REFINE THE USE OF ANIMALS

Alternatives, as articulated in *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), are defined as methods that **R**eplace, **R**educe and **R**efine the use of animals. In addition to these *Three Rs*, the Department of Defense (DoD) advocates a fourth *R*, "Responsibility," for implementing these alternative methods.

Department policy with regard to animal alternatives is promulgated in DoD Directive 3216.1 which directs that "it is DoD policy that...alternatives to animal species should be used if they produce scientifically satisfactory results...." This policy is implemented in the Joint Service Regulation on the Use of Animals in DoD Programs, which delegates responsibility to the local commander for utilization of alternatives to animals.

To illustrate the Department's initiatives to promote these *Four Rs*, a description of such initiatives within DoD's research laboratories and medical treatment centers is provided. The following list is not all inclusive, as the number of specific examples of implementing alternative methods that can be documented for DoD's research projects is large. Rather, it illustrates the scope, diversity, and spirit of DoD's *Four Rs* initiatives. This section will demonstrate a broad-based movement, where feasible, toward the use of biotechnology and other innovative adjuncts to replace and reduce animal use as well as refinement in methods used in essential animal studies.

## VI.1 RESPONSIBILITY

The DoD has established a variety of initiatives and targeted programs that are currently in place to promote alternative methods that will refine, reduce and replace the use of animals. These programs are designed to target individual and institutional awareness by providing educational opportunities, professional training and fiscal resources toward implementing the *Four Rs* approach to animal use.

### VI.1.1 Science and Technology Emphasis on Alternatives to Animal Subjects of Research

The Department of Defense continues to seek alternatives to animal use through an Army Science and Technology Objective (STO) initiated in FY 1993 and continuing through FY 2001 entitled *Reduced Reliance on Human and Animal Subjects of Research and Improving Experimental Conditions Using Animals*. The objectives of the program are to develop technologies to incrementally reduce future reliance on animals in research by 25% using FY91 as a base year, and to introduce a minimum of one improvement (methodology or technology) per year in experimental protocols using animals. The U.S. Army Medical Research and Materiel Command (USAMRMC) budgets approximately $550,000 per year for this objective which is available to support alternatives to animal use research in all three services. Recent accomplishments have included incorporation of a tumor cell screening test, based on a National Institutes of Health model, for animal toxicity testing; and, the development of computer-modeled structural mutants of various toxins for screening as medical countermeasures. Efforts are in place to evaluate *in vitro* organ slice methods to replace animal testing for toxicity, and to establish and maintain advanced biomedical databases. The U.S. Army Biomedical R&D Laboratory of the USAMRMC manages a diverse research program in the development of alternative toxicity assessment methods in collaboration with the National Institute of Environmental Health Sciences, academic institutions and the private sector. Accomplishments in this program have included the development of a new non-mammalian development toxicity model, the establishment of a cooperative research and development agreement on new non-mammalian toxicity models with Colorado State University, and representing the Department of Defense on the Interagency Coordinating Committee on the Validation of Alternative Methods in toxicity testing.

The Army STO structure provides guidance, means, and high visibility to major Army technology initiatives. The Department of Army, in coordination with the Director of Defense Research and Engineering, Office of the Secretary of Defense, publishes the *Army Science and Technology Master Plan* as guidance to Army laboratories and research, development and engineering centers and to non-Army organizations supporting the Army science and technology base.

## VI.1.2 Conferences and Workshops on Alternatives to Animal Use

The DoD promotes responsibility for alternatives to animal use by sponsoring formal education training programs and major meetings and conferences on the subject. In 1990, an important conference on alternatives to animal use, "DoD Initiatives in Alternatives to Animal Testing," was held at Aberdeen Proving Ground. This was followed by a 3-day symposium in 1992 entitled "Current Concepts and Approaches on Animal Test Alternatives" with 35 scientific platform sessions and 22 scientific poster presentations. This international symposium was attended by nearly 300 military and civilian scientists from four countries. Proceedings of the 1992 symposium were published in September 1993 and are available through the Defense Technical Information Center (DTIC). In addition, in 1994 a book edited by Dr. Harry Salem entitled "Animal Test Alternatives" was published by Marcel Dekker, Inc., which included chapters prepared by most of the presenters at this symposium (Appendix N).

The Department's continuing commitment to promoting responsibility for alternatives to animal use, even in an environment of constrained resources, is reflected by another such conference held on 24-26 May 1994, at Aberdeen Proving Ground entitled "Alternatives in the Assessment of Toxicity: Theory and Practice" (Appendix O). This international conference with 26 scientific platform sessions, including one by Dr. Martin Stephens of the Humane Society of the United States, and 45 scientific poster presentations was attended by over 330 military and civilian scientists from seven countries. The proceedings and a monograph based on this successful symposium are available through DTIC. The book "Advances in Animal Alternatives for Safety and Efficacy Testing" is being published by Taylor and Francis (Appendix N). This symposium and the 1994 one were praised as a success by Dr. Martin Stephens of the Humane Society of the United States (Appendix P). A 4th Biennial International Symposium on Alternatives in the Assessment of Toxicity Issues, Progress and Opportunities was held 12-14 June 1996 at the Aberdeen Proving Ground-Edgewood Area, Maryland (Appendix O). This DoD conference was coordinated with the Scientists Center for Animal Welfare who hold their meeting 10-11 June 1996 to present Animal Welfare and Toxicology/Safety Studies: Current Issues and Trends for the Next Century. Thus a full week in Maryland was devoted to discuss Animal Welfare and Alternatives in Toxicology and Safety Studies.

DoD is also represented on the Interagency Regulatory Alternatives Group which planned and presented a "Workshop on Updating Eye Irritation Test Methods" in 1991 and held another workshop on Dermal Testing held at the American College of Toxicology, in November 1995. The National Institute of Environmental Health Sciences has established the Interagency Coordinating Committee on the Validation of Alternative Methods in response to the Revitalization Act of 1993, which also has DoD representation. Presentations have also been made on alternatives to the Board of Scientific Councilors of the National Toxicology Program of the National Institute of Environmental Health Sciences (NTP-NIEHS), Board of Scientific Councilors of the Food and Drug Administration and Cancer Etiology Group at the National Cancer Institute.

## VI.1.3 National Research Council, Institute of Laboratory Animal Resources, Educational Programs

The DoD's priority and continuing commitment to promoting individual and institutional responsibility for alternatives to animal use are reflected in continuing financial support of the Institute of Laboratory Animal Resources (ILAR) educational program of the National Research Council. The principal thrust of the ILAR grant is development of institutional training materials, educational courses and publications in support of the Department's laboratory animal care and use programs. This ILAR information is used in various military research facilities as an important adjunct

to existing investigator training and technical education programs on animal care and use. The ILAR information and programs have generated strong animal alternative provisions for military-specific research. The Department previously funded a 5-year ILAR grant (DAMD 17-87-G-7021) for this program and is currently in the third year of another 5-year ILAR grant (DAMD 17-93-J-3016) committing diminishing research funds to maintain this important collaboration. Annual funding for this DoD-sponsored ILAR program is in excess of $100,000. In addition, National Research Council fellowships for conducting research in alternatives to animals are available at the U.S. Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Maryland (Appendix Q).

### VI.1.4 Institutional Animal Care and Use Committee Emphasis

Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1-4 of the Code of Federal Regulations has specific provisions for addressing the issue of alternatives during the research animal protocol review process. The DoD has been a leader in forming lawfully constituted and functioning Institutional Animal Care and Use Committees (IACUCs) at its biomedical research facilities. Accordingly, DoD IACUCs consider alternatives to the proposed use of animals as an important review consideration. All DoD programs use a Standardized IACUC Protocol Format for animal use proposals, which requires that non-animal alternatives be considered. It states that "No study using animals should be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means." Investigators must provide information on the animal model being proposed and justification for the selected species. The Standard Protocol Format states that "investigators should use the least sentient species that will permit the attainment of research objectives." In addition, the investigators are required to provide a short description of the features of the proposal that may qualify the study as one that refines, reduces or replaces the use of animals. The DoD 1995 Policy letter requires that extramural contractor proposals utilizing animals in research, testing or training include all the information contained in the DoD Standard Protocol Format, thereby requiring them to also provide the alternatives information.

### VI.1.5 Veterinary Staff Expertise and Assistance Visits

The major biomedical research commands of the Military Departments each have credentialed laboratory animal medicine (LAM) veterinarians serving in key staff positions. Approximately 5% of the board-certified specialists of the American College of Laboratory Animal Medicine (ACLAM) currently serve in the DoD. In addition to being advisors to commanders on issues related to animal welfare and alternatives to animal use, these veterinarians provide oversight and structure to the command's animal care and use programs. These officers also make periodic staff assistance visits to subordinate facilities that use animals and evaluate each laboratory animal care and use program. Consideration of the use of alternatives is reviewed on these staff assistance visits. Another important responsibility of the LAM veterinarian is to review extramural animal use protocols, ensuring that alternatives to animal use and personnel training issues have been addressed.

### VI.1.6 Professional Veterinary Training in LAM

The individuals who are specialty trained in veterinary Laboratory Animal Medicine provide expertise in DoD biomedical research institutions which strongly correlates to effective animal use alternatives programs. This is especially true in the critical area of refinements. The DoD has long been a leader in training veterinarians in the field of LAM, the biomedical and veterinary specialty most closely associated with laboratory animal welfare and laboratory animal care and use programs. Many of the nationally prominent leaders of several laboratory animal associations were formally trained in, or closely associated with, DoD LAM training programs. Examples are the President-elect and several past presidents of ACLAM, the President and several past presidents of the American Association of Laboratory Animal Science (AALAS), and several past presidents and the current Secretary-Treasurer of the American Society of Laboratory Animal Practitioners. This traditional DoD strength in LAM expertise strongly enhances both animal care and use and animal

alternatives programs. Approximately 25% of all ACLAM boarded specialists in the U.S. received some or all of their LAM training in DoD LAM training programs.

### VI.1.7 AALAS Technician and Laboratory Animal Science Training

There are a number of DoD research facilities that sponsor formal training programs leading to certification of animal care and research personnel as AALAS laboratory animal technicians. This specialized training is offered to both government and non-government animal technicians. It is an important mechanism for ensuring highly qualified animal care and research technicians in Defense laboratories. Individual DoD institutions have sponsored formal seminars for research personnel where experts from the National Agricultural Library, Animal Welfare Information Center explain in detail the resources available for exploring various animal alternatives in the laboratory. The Walter Reed Army Institute of Research (WRAIR) sponsors laboratory animal workshops that provide comprehensive technical training available to all DoD personnel on animal use and related issues. Improving the technical expertise of laboratory animal technicians and investigators is a significant refinement element for the use of animals in the laboratory. These workshops are available to all DoD and National Institutes of Health laboratories. As an example, the workshop on the use of rodents is offered 14 times per year. In addition, WRAIR offers quarterly a workshop on ethical and administrative issues relating to animal use. The AALAS technicians' course curriculum and the WRAIR workshop curriculum include formal training and information on alternatives to animal use.

## VI.2 DoD Initiatives to Replace, Reduce and Refine the Use of Animals

The following specific examples are a representative listing of alternative methodologies practiced in DoD facilities. They are categorized as Replacement, Reduction, and Refinement initiatives. Because of the multifaceted aspects of many of these examples, some logically belong in more than one category. Alternative methodologies with an asterisk (*) indicate an alternative first reported in FY95 by a DoD facility or extramural contractor. Examples with a bullet (•) indicate an alternative reported in FY93 and FY94.

### VI.2.1 Replacement

The replacement alternative addresses supplanting animal use with non-living systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. Additionally, human subjects are used when experimental drugs and other procedures progress to human trials. Such trials are conducted in accordance with Title 32, U.S. Code of Federal Regulations, Section 219, "Protection of Human Subjects in DoD-Sponsored Research."

### VI.2.1.A Replacement Using Biochemical or Physical Methods

- Membrane feeding systems have been developed that replace the need to feed some types of blood-feeding flies and mosquitos on rodent hosts.
- Development of Polymerase Chain Reaction (PCR) and Mammalian Cell Selection Assays for short-term genetic toxicity testing replaces animal use in carcinogenesis and mutagenesis studies.
- Efforts are ongoing to develop a PCR assay for Q-fever that could eliminate the need for the use of a mouse bioassay.
- Use of PCR for assessment of viral infections.
- Quantitating bacterial endotoxin with an *in vitro* Limulus Amebocyte test replaces *in vivo* pyrogen testing in rabbits.
- Use of predictive anthropomorphic dummies and manikins, e.g., ADAM (ejection seat reactive live load manikin) and AIRMAN (a fragment capture live fire manikin) has replaced the use of animals in these studies.

### VI.2.1.B Replacement Using Computer Simulations

- Computer models to replace rhesus monkeys and baboons for toxicological studies are being developed.
- Development of computational models of dolphin echolocation (sonar) for inclusion in the development of hardware systems will replace use of animals as object detectors.
- Development for Special Forces medical training personnel of advanced computer technology using Virtual Reality, Holographic Imaging, and Telepresence Surgery techniques may replace the use of animals in Special Forces surgical training.
- Computer models are being developed for predicting carcinogenesis induced by ionizing radiation replacing the need to use animals.
- A computer model for predicting the transfer of toxic chemicals across the intestinal mucosa and into the blood stream is in development.

### VI.2.1.C Replacement Using *in vitro* Cell Culture

* Replace mice by use of bioreactor to grow large volumes of specific antibody.
* Cell cultures are used to replace mice and rats to test inhibitors of cAMP degradation.
* Subunits of AMPA receptors have been stably expressed in a cell. A significant portion of this study concerning drug effects of AMPA receptor physiology and ligand binding are now carried out in these cell lines.
* Use of P450 isoenzymes to develop metabolism assays.
* Limulus amchocyte lysate assay has been used to test for endotoxin in vaccines.

- *In vitro* cell culture methods have been developed for passage of Hepatitis E virus eliminating use of most animals for virus propagation.
- Development of a macrophage cell line to replace animals in evaluation of cytotoxicity and genotoxicity of respirable particles is in progress.
- Development of a fish liver cell culture model for evaluating metabolism of Xenobiotic compounds replaces the use of mammalian animal models.
- Tissue culture using human gingival fibroblasts replaced the need to use rats to study the effects of Transforming Growth Factor-Beta (T6F-B) on wound healing.
- Cell cultures are being evaluated to replace mice as a host assay for detecting and identifying anthropod-borne viruses.
- Use of a rat cell line obtained from the American Type Culture Collection to study Calcium Channel Blockers and Angiotensin Converting Enzyme Inhibitors eliminates the use of pigs.
- Established cell lines from American Type Culture Collection are used in place of mice to test the effects of antibiotics on cell proliferation and inhibition of DNA synthesis as well as to test the effects of anti-progesterone chemicals on proliferation and/or inhibition and tumor cell death.
- Established cell line of macrophages from American Type Culture Collection to study the alteration of macrophage chemotactic response by oxygen replaces the use of mice and rats.
- Development of an *in vitro* hepatotoxicity screen to rank order chemicals for their ability to damage the liver will replace the use of mice and rats.
- Use of human mononuclear cells analyzed by flow cytometry to determine expression of CD69 after staphylococcal enterotoxin B

(SEB) treatment, may replace the use of mouse spleen cells.

- Cell and organ cultures to replace the rabbit for mucin-type glycoprotein in malignant breast tissue studies.

- Cell and organ cultures to replace the rat in regulated mucin gene expression in airway injury studies.

- Use of human and animal peripheral blood lymphocytes with flow cytometry to assess cytotoxicity and DNA alterations induced by sulfur mustard and its monofunctional analogue chloroethyl-ethyl sulfide replaces the use of hairless guinea pig and weanling domestic swine.

- Human cortical cell lines (HCN-1A) were used instead of rats to determine specific characteristics of sodium channels, in an effort to confirm their usefulness in studying toxins that produce their effect by sodium channel blocking.

- The HeLa Cell, a human epithelial tumor line, has been established as a useful proliferating cell model in sulphur mustard studies, replacing the use of hairless guinea pigs and weanling domestic swine.

- A terminal deoxynucleotide transferase assay was developed to measure the presence of DNA single strand breaks following sulfur mustard exposure of peripheral blood lymphocytes and human epidermal keratinocytes, replacing the use of hairless guinea pigs and weanling domestic swine.

- Human epidermal keratinocytes cultures are used as a model system to study the change in poly (ADP-ribose) polymerase (PADPRP) activity levels following sulfur mustard exposure, replacing the use of hairless guinea pigs and weanling domestic swine.

- Studies with neuroglioma cells in culture (NG108-15 cells) suggest that they are acceptable for validation of the PADPRP assay and for preliminary concentration dose response curves of sulfur mustard-induced cytotoxicity and PADPRP activity, replacing the use of hairless guinea pigs, rats, mice, and guinea pigs.

- Cells are exposed to dilute liquid sulfur mustard. At selected intervals post-exposure, samples are prepared for either biochemical or ultrastructural analyses. Analysis includes the application of specific probes (e.g., antibodies) or gel electrophoresis and electron microscopic autoradiography. Once identified, specific molecular targets are developed for use as biological markers in antivesicant drug assessment. These techniques will replace the use of hairless guinea pigs and guinea pigs.

- Living "TESTSKIN" (the commercial human skin equivalent) cellular models are used to elucidate the biochemical mechanisms responsible for sulfur mustard-induced pathology, replacing the use of hairless guinea pigs and weanling domestic swine. As the mechanisms are defined, studies of therapeutic intervention are evaluated for protection against sulfur mustard-induced pathology.

- A contract with the Cooperative Human Tissue Network provides human skin biopsies that replace the use of hairless guinea pigs and weanling domestic swine. Techniques for explant culture were developed and the specimens evaluated for histologic integrity over the first 5 days following receipt.

- Transendothelial electrical resistance and ultrastructure of cultured bovine pulmonary endothelial cells are determined after direct exposure to three edemagenic gases: phosgene, perfluoroisobutylene, and bis (trifluoromethyl) disulfide. Membrane electrical resistance is a sensitive method of determining tissue integrity and can be used to assess changes in cell-to-cell interactions that affect permeability of the endothelial barrier. These techniques replace the use of rats, guinea pigs, and sheep.

- Clonal neurosecretory cells of adrenal chromaffin or clonal pheochromocytoma origin are individually injected with botulinum toxin or the purified light chain of botulinum toxoid. Patch clamp recordings are used to measure capacitance changes associated with fusion of neurosecretory vesicles with the plasma membrane. Detailed examinations of membrane events during vesicle fusion are performed in the presence and absence of botulinum toxin. These techniques replace the use of mice, rats, and guinea pigs.

- Clonal neurosecretory cells of adrenal chromaffin or clonal pheochromocytoma origin are transfected with antisense oligonucleotides to suppress protein production from specific mRNAs. Secretion of the vesicle contents in response to potassium stimulation is then measured to assess the importance of the suppressed protein to synaptic transmission. These techniques replace the use of mice, rats, and guinea pigs.

- Study of the effects of growth factors on human fibroblasts is being conducted in cell culture media replacing the dogs and pigs utilized in previous studies.

- Development of a cell culture system to pass human breast cancer cells eliminates the need for initially passing these cells in a nude mouse model.

- Use of immortalized tissue culture systems or isolated lobster neuronal cells to investigate radiation effects and free radical damage to the nervous system at the molecular level are used to replace similar protocols using rats and guinea pigs.

- Wound-healing studies on space shuttle flights STS-45, 55 and 56 used a cell culture flight module instead of live rats.

- Development of human skin cell and animal processing plant skin models for assessing cellular mediator and tissue damage from environmental heat has replaced mammalian laboratory animal use.

### VI.2.1.D Replacement with Non-Mammalian Species and Species Lower in the Phylogenetic Scale

* Use of invertebrate sea slug (*Aplysia californica*) to study effects of chemicals on electrical properties of nerve cells to replace mammalian laboratory animals.

* Use of guinea pigs will preclude the requirement for nonhuman primates in all but the most critical pathogenesis and protective efficacy studies.

* Animals that are low on the phylogenetic scale (mice) are being used to determine the optimum dose schedule, route of immunization and other parameters of vaccine development. This study minimizes use of nonhuman primates.

* Replace nonhuman primates with rats for Hepatitis E Virus bioassay.

* Mice are used instead of nonhuman primates in newly developed test for Toxic Shock Syndrome Toxin 1.

* Nonhuman primates are replaced with guinea pigs to test immune response to Shigella vaccines, and in the Leishmania Skin Test.

- Development of an aquatic bioassay using the medaka fish (*Oryzias latipes*) to assess human carcinogenic health risks replaces laboratory animal use for tumor immunodiagnosis.

- South African clawed frog (*Xenopus laevis*) embryo replaces laboratory mammals commonly used in teratogenesis assays and in neurotoxicology research.

- Aquatic organisms (Japanese medaka, zebrafish, bluegill, guppy) replace mammals commonly used in toxicology research.

- Rats and swine may replace cynomologus monkeys as an alternative model for hepatitis E.

- Pigs used in emergency room and surgical resident training; and hamsters, rabbits, pigs and rats in veterinary proficiency training to replace dogs.

- Ferrets used in pediatric advanced life support courses and endotracheal intubation exercises to replace cats.

- Development of genotoxicity model using fish as an alternative to the conventional rodent model.

- Cardiopulmonary measurements previously conducted in monkeys and guinea pigs are now carried out in free-moving unrestrained rats.

### VI.2.1.E Replacement with Human Tissue, or Volunteers as Protocols Progress to Human Trials

* Use of cytosensor microphysiometer which utilizes human cell lines to assess both acute and chronic toxicity. Replaces use of laboratory mammals.

- Many procedures including conjunctival impression cytology, salt and water balance and intestinal permeability, neuroendocrine assessment, nutritional support, testing of topical treatments and studies of *in vitro* activated keratinocytes in autografts in thermal injury research were previously performed in animals but have now progressed to human use protocols, eliminating the use of animals.

- Biomechanical analysis of the strength of plate fixation devices for long bone fracture repair is being performed with human cadaver bones and metal substitutes thereby replacing animal studies.

### VI.2.1.F Replacement with Discarded Tissue from Other Laboratories or Food Processing Plants

- Pigs feet obtained from a local plant are used for teaching surgical suturing procedures, replacing the need for use of live animals.

- Sheep parts purchased from a processing plant are used to train dentists on periodontal surgical procedures replacing the use of live animals for training.

- Ocular researchers are using eyes purchased from local cattle processing plants for studies instead of live rabbits.

- Training programs for urology residents utilizing lasers for bladder treatments are initially performed with pig bladders purchased from a processing plant. This reduces the number of animals used for surgical training.

- Evaluation of suture patterns and angioplasty balloons on vein graft anastomosis on pigs used for surgical procedure training. Sharing of animals reduces the total number of animals used.

### VI.2.2 Reduction

Decreasing the numbers of animals used through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

### VI.2.2.A Reduction by Use of Alternative Screening Methods to Study Efficacy in Biological Testing

- Development of a Quantitative Luminescence Imaging System for screening radiofrequency radiation biological effects in cells reduces the number of laboratory animals needed.

- Establishment of a tissue culture system to evaluate initial exposure levels of toxic substances, such as ammonia, or nitrogen and sulfur oxides, in lung and throat secretions reduces the use of animals in subsequent therapy studies.

- Development of an *in vitro* test using human peripheral blood could determine the effectiveness of toxoid in a SEB vaccine and measure the effectiveness of potential

treatments to SEB poisoning. If validated, this would significantly reduce the animals used in SEB research.

- Use of bacteria, algae, crustaceans, earthworms, flatworms, and a toxicity estimation software program functions as a screening mechanism in toxicity testing, highlighting those chemicals or materials necessitating further testing with fish or higher vertebrates. This eliminates many compounds from further testing and reduces laboratory animal use.

- Use of cell culture or molecular biology in preliminary studies of basic mechanisms of cardiovascular disease. An example is the use of an immortal cell line in molecular research on the effects of oxygen on the chemotactic response of macrophages to oxygen, reducing the need for whole animal studies.

- Development of fish (rainbow trout, zebra danio and medaka) as predictive models for epigenetic carcinogens has reduced mammalian animal use in carcinogenesis studies.

- Development and validation of fish immune responses as a biomarker to replace laboratory mammals.

- Purchase of elutriation system reduced the number of mice required for Modulation of Kupffer Cell Tumoricidal Properties by 50%.

- Toxin and toxoid preparations are titrated in a newly developed cell assay to minimize the use of animals for dose determination.

- Development of an *in vitro* test for cytoadherence by malaria-infected erythrocytes to human melanoma cells, umbilical vein cells, and endothelial cells greatly reduces the need for nonhuman primates.

- Development of a severe combined immunodeficiency disease mouse model where transplanted human liver tissue, a target for malarial sporozoite infection, cannot be rejected, permits the evaluation of potential malarial vaccine candidates in a non-monkey model.

- Development of an *in vitro* drug screening system using infected human cells to replace the mouse malaria lethality model, eliminating the need for 4,000 mice per year.

- *In vitro* drug screening, drug release kinetics, etc., result in reduction of drug candidates for numerous toxins reducing *in vivo* testing in rodent models up to 90% in some studies.

- Significant effort to develop DNA probes to detect *Orientia tsutsugamushi* in mammalian (including human) and chigger tissues should result in a 50% decrease in animal use for isolation and detection of this infectious agent.

- Development of an *in vitro* cultured human hepatoma cell line to assess radical and curative prophylactic activity of antimalarial drugs is in progress. This has the potential to reduce the number of monkeys needed for assessing antimalarial drugs and related compounds.

- *In vitro* techniques using human bone marrow cell culture to demonstrate propagation of Dengue viruses in these cells have reduced the number of monkeys needed for viral propagation by 25%.

- Development of a mosquito model using *in vitro* Dengue antigen detection techniques to pre-screen Dengue candidate vaccines should reduce the number of nonhuman primates needed for evaluation of vaccine candidates.

- Development of a reliable cell culture system for evaluating *Orientia tsutsugamushi* antibiotic resistance has reduced the need for animals for drug resistance studies by 50%.

- DNA probes have been developed to screen human *E. coli* isolates for pathogenicity. Only those positive to *in vitro* screening are tested in animals to confirm pathogenicity;

this greatly decreases the numbers of animals used.

- Use of ELISA (enzyme linked immunosorbent assay) tests as a first screen in cellular mediator (interleukin 1) studies has reduced the number of mice previously required by 90%.

- The nervous systems of invertebrate sea slugs are used to study the effect of chemical and toxic agents on the electrical properties of nerve cells. This preliminary work reduces the number of vertebrates needed for subsequent study.

- Development and use of amphibian models (*Xenopus laevis* - frog) for assessing teratogenesis assays significantly reduce mammalian animal use.

- Interlaboratory validation of the Frog Embryo Teratogenesis Assay in collaboration with NTP-NIEHS. On-going work with NTP-NIEHS to develop non-mammalism alternative methods for neurobehavioral and reproductive toxicology endpoint assessments. Collaborative work with NIEHS to use genetically engineered fish to investigate the effects of environmental contamination.

### VI.2.2.B Reduction by Substitution of *in vitro* or *ex vivo* Methods

* Numbers of mice and rats reduced to test inhibitors of cAMP degradation. Instead of 1 mouse and 1 rat per assay, 50 can be assayed from cell cultures made from 1 mouse or 1 rat.

* Reduction in numbers of swine and goats by conducting power analysis to determine minimal numbers of animals to use in surgical studies.

* Reduction in numbers of swine and goats in ocular studies by using same animal for both test and control eyes.

* Reduce number of nonhuman primates and rats by focusing on selected endpoints for limited defined periods of time.

* Reduction in use of nonhuman primates for testing SEB toxoid vaccines. Mice are used for screening, safety testing, immunogenicity evaluation, and challenge studies utilize the same nonhuman primates instead of additional animals.

* Placing stents in both femoral arteries of rabbits allow each animal to serve as its own control.

* Cell culture effort reduces number of rats, sparrows and chickens used in basic research.

* Swine from other training protocols were euthanized and the eyes collected for other studies.

* Investigators share tissues from same experimental animal (cotton rat) allowing for reduction in the number of animals utilized.

* Candidate vaccine was tested *in vitro* in lymphocyte proliferation assay and receptor binding assay in both rodent and human cell lines.

* Screening of antibiotics for *in vitro* activity reduces number of animals needed.

* A nuclear magnetic resonance technique reduced the number of rats needed in a study since each animal provides data over many time points.

* Vaccine efficacy studies in rabbit provided better predictive data for range finding and thus reduce numbers of nonhuman primates.

- Synthetic *in vitro* or *ex vivo* systems like artificial bimembrane layers, cell or tissue culture systems, and isolated diaphragm

muscle preparations replace or reduce the need for live, whole animal experiments in medical chemical defense research.

- Perfection of an *in vitro* method for growing *Plasmodium falciparum* (the most important human malaria that affects only man and certain monkey species) in human red blood cells has greatly reduced the number of nonhuman primates needed for this research.

- Development of specialized insect and vertebrate cell lines have reduced the need for intracerebral inoculation of suckling mice for the isolation of arboviruses.

- Use of transformed (immortal or self-propagating) cell cultures as an alternative to primary cell cultures that require frequent harvesting of tissues from animals.

- The use of monoclonal antibodies from hybridoma cells to replace animal-derived polyclonal antibody preparations greatly reduces animal requirements.

- Tissue culture of mouse osseous cells used as a reduction strategy for live animals to study biocompatibility of dental impression materials.

- *In vitro* techniques to orally infect mosquitoes with Dengue viruses have reduced the number of mice and monkeys needed for viral propagation by 25%.

- Development of new technology utilizing tissue slices from dead animals to assess the toxicity of selected environmental contaminants.

- Use of isolated perfused liver preparation to study the hepatotoxic effects of selected chemicals.

- Use of cultured cells for cytochrome P450 induction in vertebrate endothelium. Cells from 6 pigs represented the equivalent of approximately 100 pigs for *in vivo* studies.

- Cell cultures being developed to study mechanism of cyclic hydrocarbons and heavy metal toxicity.

### VI.2.2.C Reduction by Substitution of Another Animal Species, or Human Subjects as Protocols Progress into Human Trials

- Studies have been performed to develop mouse and guinea pig models to replace the monkey as an aerosol model for botulism, staphylococcal enterotoxin B, and plague intoxication, which greatly reduces the number of monkeys needed for biological product toxicity and protective efficacy testing.

- Progression of a model of anti-malaria protective immunity into humans, where protective immunity is induced in human subjects by injected irradiated malarial sporozoites, has reduced the need for animal use in malaria research.

- Although cynomolgus monkeys are the only known model for Hepatitis E infection, rats, lesser bandicoots (rat-like animal) and swine are being evaluated as alternative models to reduce the need for monkeys.

### VI.2.2.D Reduction by Substitution of Computer Simulations or Other Technologies

* Reduction in numbers of mice, rats and guinea pigs by using radiology and assays.

* Reduction in numbers of mice through use of computer modeling of potential peptide antigens to determine if conformation sequence is analogous to native protein.

* Biostatistical review for research design to ensure the minimal, yet statistically significant number of animals were used.

* Hamsters from one study were reused in another study.

* The use of historical control data instead of animals reduces number of nonhuman primates needed in studies of vaccine efficacy.

* Several groups of mice were tested concomitantly so fewer control animals were needed.

- Use of bioengineering tools to measure physiological parameters on human subjects in operational and experimental gravity tolerance environments may result in a decrease in the number of animals currently used in gravity tolerance work.

- A research effort is aimed at developing physiologically based computer models/ algorithms to predict *in vivo* distribution, uptake, and elimination of toxic chemicals, thus reducing the need for animals.

- Development of a computer model simulating *in vivo* absorption, distribution, metabolism, and toxic effects of nerve agents and vesicants and validated against *in vivo* pharmacokinetics data in guinea pigs for the nerve-agent soman will significantly reduce the number of animals used in nerve-agent research.

- Training of professionals by interactive videos and innovative teaching techniques, e.g., laparoscopic instruments on synthetic sponges, reduces the use of animals.

- Integration of mathematical modeling and aeromedical cardiovascular nonhuman primate research should reduce animal use.

- A computer-modeling program reduces the use of sheep in blast overpressure research.

- A computer-modeling program that identifies active sites on large molecular weight toxin molecules for intervention with therapeutic drugs is underway. This effort will substantially reduce the numbers of animals used in biotoxin studies.

- Development of a model to understand the propagation and bioeffects of electromagnetic energy should reduce the number of animals used.

- Physiologically based pharmacokinetic modeling to predict toxicity and metabolism of trichloroethylene, vinyl chloride and their mixtures, by oral and inhalation routes reduces the use of mice and rats.

- Development of a computer model to predict the distribution and toxic effect of candidate replacement fire extinguishing agents. This technique will reduce the use of rats.

### VI.2.2.E Reduction by Sharing Animals between Research Investigations

- Use of the same control animals for more than one protocol reduces the number of animals required.

- By combining anesthesia and surgical demonstrations in goats, the numbers were reduced from eight to four.

- Military working dogs scheduled for euthanasia are used for training labs, while under anesthesia.

- Guinea pig tissue, required for an improved histology method for hydration and preservation of tissue morphology, is taken from guinea pigs used in other projects. Since animals are used twice, it reduces the total number of guinea pigs used per year.

- The effect of magnesium on ventricular rate control during a trial fibrillation was studied using pigs transferred from another protocol. The re-use of swine reduced the total number of swine used per year.

- Temperature monitoring during craniotomy procedures was carried out in conjunction with another protocol requiring swine. Re-use of swine reduced the total number of swine used per year.

- Training in trans-septal right heart catherization utilized sheep being euthanized as

part of another protocol. Re-use of sheep reduced the total number of sheep.

- Hearts from rats used in other experiments were utilized in studies of Growth and Characterization of Rat Cardiac Myocytes in a Capillary Cell Culture System - on Earth and in Space.

- Gastrointestinal tracts from baboons used in experiments at an independent research foundation were obtained and used in Postnatal Gastrointestinal Adaptation in Extremely Preterm Baboons with Respiratory Insufficiency: Effects of Trophic Feeds.

### VI.2.3 Refinement

The refinement alternative for animal use addresses the need to ensure that the maximum humane use of each animal is obtained through proper protocol design and efficient utilization of animals, or through the modification of the experimental design to reduce the ethical cost associated with the study.

### VI.2.3.A Refinement to Protocols that Reduce Pain

* Refinement reduces pain in pigs and rabbits used for surgical training of physicians by adding long-acting local anesthetics in addition to general anesthesia.

* Refinement in experiments on pigs assures maximal utilization using very sophisti–cated instrumentation.

- *Ex vivo* cardiovascular response studies (using tissues in isolated systems) of toxins eliminate potential pain and distress for animals that would be used in whole animal systems.

- Refinement of methodologies associated with the feeding of arthropod vectors (chiggers) on rodents reduces discomfort to the animals. Use of an unobtrusive barrier system to prevent escape of the chiggers eliminates the need for the attachment of a cumbersome feeding capsule on the anesthetized animal.

- Studies performed to compare less reactogenic adjuvant regimens and alternative sites to foot pad injections in guinea pigs for evaluating hypersensitivity reactions (inflammation and swelling) from candidate Q–fever vaccines decrease potential discomfort associated with evaluation of vaccine candidates.

- Sophisticated technology such as nuclear magnetic resonance imaging is used to follow biochemical changes occurring over time in rats and other animals. This non-invasive procedure results in the use of far fewer animals and a more physiologically normal model.

- Development and evaluation of micro-encapsulated, time-released anesthetics and analgesics potentially beneficial to casualties on the battlefield have been performed. If perfected, these compounds will provide long-acting analgesia or anesthesia for animals on research projects where anesthesia or analgesia is not currently feasible.

- An evaluation of the feasibility and effectiveness of using topical analgesia (pain relief) on rabbits in Draize eye irritancy testing, and in systemic analgesia during Sereny' Testing (inflammation bioassay) on guinea pigs was performed. This provides the ability to perform a test while decreasing pain and distress without altering the outcome.

- A transdermal (applied to the skin) delivery system of analgesia to relieve pain in dogs was evaluated. It provides an extended analgesia or anesthesia for animals on research projects, and will be of benefit in human and veterinary medicine for the relief of pain.

- Use of long-acting local anesthetic in addition to general anesthesia and post-op analgesics to relieve pain in graft adhesion studies in rabbits, pericranium tissue barrier

in mandibular reconstruction studies in sheep, and pleurodesis by thoracoscopic microfibrillar collagen studies in the pig. A specially designed sling was used in the pig studies.

- In rabbit studies of repair of abdominal rectus fascia, long-acting post-op analgesics are used to reduce or eliminate pain.

## VI.2.3.B Refinement to Protocols that Reduce Distress

* Refined technique for hypothermia experiments in microswine using general anesthesia. Environmental enrichment strategies to reduce stress included mineral oil rubs and the availability of "play" objects.

* Use of a slow release subcutaneously placed estrogen capsule avoids the need for daily intramuscular injections in rats.

* Percutaneous techniques used for carotid artery access avoid the use of large surgical cut-down procedures on femoral arteries in rabbits.

* Use of warming blankets and improvements in post-operative positioning of animals has improved post-operative recovery of rats and rabbits.

* Animals were acclimated and trained to minimize pre-phlebotomy stress.

- Development of telemetric surgical procedures for implantation of sensors, allows non-stressful measurement of clinically relevant physiological parameters in non-clinical vaccine and drug efficacy studies. This not only decreases stress associated with manipulative measurements, but the radio-transmitted measurements vastly improve the quality and quantity of data available. Additionally, use of the telemetry allows physiological assessment for efficacy trials, makes intervention with analgesia more feasible, and significantly reduces the use of lethality as the primary endpoint.

- Video tapes are used for adjunct training of technicians and investigators for common animal use procedures, i.e., venipuncture, handling, and restraint.

- Novel antibody production and collection techniques in rabbits and goats with plasma collection chambers reduce potential distress associated with venipuncture procedures and reduce, and, in some cases, eliminate immunoadjuvant use.

- Use of slings for studies requiring restraint of pigs and extensive conditioning of the swine prior to initiation of the study result in a significant refinement by reducing potential distress.

- DoD facilities use social housing systems, e.g., multiple animal housing or gang caging, where feasible, which expand intraspecies interactions, and use environmental enrichment strategies that extend to many species that are not specifically mandated by animal welfare legislation. These housing strategies increase the quality of life for the animals.

- A flexible polyethylene mesh restraint device that is more comfortable and is well tolerated by rodents replaces the use of rigid restrainers previously used for maintenance of arthropod (mosquito) vectors.

- A project is underway that plays back natural nonhuman primate vocalizations and analyzes the effectiveness of this as an environmental enrichment strategy.

- A hyphema (fluid in the anterior chamber) model in rabbits has been developed using a non-invasive laser beam to open intraocular vessels and to create the hyphema instead of the standard surgical procedure previously required. This procedure eliminates post-surgical distress.

- Study endpoints are adjusted to reduce the need to proceed to death as a defined protocol objective. An example is the evaluation of the neurotoxicity of candidate therapeutic radioprotective compounds in

mice using decrements or changes in motor behavior and coordination as a definitive endpoint rather than death. Another example is using respiratory distress, rather than death, as an endpoint in the *in vivo* Study of Enhancement of Cis-Platinum Antitumor Activity by Pentoxyfyllin in Nude Mice with Human Ovarian Carcinoma.

- A non-lethal model of botulism that detects intoxication by sciatic nerve paralysis in mice is under development and will be a significant refinement to the current mouse bioassay.

- By increasing quarantine time by at least a week for goats used for training, stress-related illness and deaths were decreased.

- By creating a carotid loop, the hemodynamics of simulated amniotic fluid embolism could be studied on unanesthetized sheep with minimal restraint.

- Comparison of metabolic constants for halocarbons derived from animal studies can be used to enhance the predictive value of human *in vitro* data in the risk assessment process.

- Comparison of *in vitro* results using tissues derived from the same animal to help validate the *in vitro* assay as an alternative to live animal use in toxicology research.

### VI.2.3.C Refinement in Research Models and Animal Alternatives

- Professional biostatisticians are used by IACUCs to collaborate with scientists on experimental design and to review proposals in committee to ensure that only the minimal numbers of animals needed for statistical validity are approved for use.

- Extensive use of purpose-bred, (e.g., nude mice, hairless guinea pigs) micro-biologically and genetically defined research animals yields better animal models and more meaningful and relevant research results.

## VI.3 Summary

Each year new techniques and capabilities improve the handling, treatment, and use of animals in research and testing, and potentially reduce the need for animals in those same endeavors. In FY95, there was ample evidence of the DoD's aggressive pursuit of alternatives to replace, reduce and refine the use of animals, for example, USAMRMC's STO on reducing reliance on animals for research and improving experimental conditions using animals, and the development of the Frog Embryo Teratogenesis Assay toxicity test. In addition to these developmental efforts, animal use data for FY95 indicate the widespread implementation of validated alternatives. Rats and mice continue to replace nonhuman primates and other mammals higher on the phylogenetic scale in vaccine and drug development efforts. These and other examples of the development and implementation of alternatives have translated into reductions in the overall use of higher animals (see Section V). Animal use alternatives including refinement, reduction, and replacement constitute key initiatives in the biomedical research, testing, education, and training programs of the Department of Defense. The number of large animals used by the military departments over the past decade has been significantly reduced, and some large species are rarely used at all. Dogs, cats, nonhuman primates, and marine mammals collectively now represent less than .7% of the total animals used in research by the DoD.

# SECTION VII
## GLOSSARY

**Adjuvant:** An agent mixed in a vaccine to enhance the immunological protection afforded.

**Alternatives to Animal Use:** For purposes of this assessment, "alternatives" are defined as encompassing any subjects, protocols, or technologies that replace the use of laboratory animals altogether; reduce the number of animals required; or refine existing procedures or techniques so as to minimize the level of stress endured by the animal. These technologies involve the continued, but modified, use of animals; use of living systems; use of chemical and physical systems; and use of computers.

**Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC):** A voluntary private organization that, by Fall 1995, provided accreditation for 604 institutions. AAALAC accreditation is based on the provisions of the NIH *Guide for the Care and Use of Laboratory Animals*, and is recognized by the Public Health Service.

**Analgesic:** An agent that relieves pain without causing loss of consciousness.

**Anesthetic:** An agent that causes loss of the sensation of pain. Anesthetics may be classified as topical, local, or general.

**Animal:** For purposes of this assessment excluding embryos, animal is defined as any nonhuman member of five classes of vertebrates: mammals, birds, reptiles, amphibians, and fish. Within this group, two kinds of animals can be distinguished, warm-blooded animals (mammals and birds) and cold-blooded animals (reptiles, amphibians, and fish). Under this definition, invertebrates are not included.

**Animal Use:** The use of animals for research purposes. Three aspects of animal use are addressed in this assessment: behavioral and biomedical research; testing products for toxicity; and education of students at all levels. This assessment does not cover animal use for food and fiber; animal use to obtain biological products; or animal use for sport, entertainment, or companionship.

**Animal Welfare Act:** This act, passed in 1966 and amended in 1970, 1976, and 1985, was originally an endeavor to stop traffic in stolen animals that were being shipped across State lines and sold to research laboratories. Amendments to the act have expanded its scope to include housing, feeding, transportation, and other aspects of animal care; however, the act bars regulation of the conduct of research and testing by USDA. Animals covered by the act, as currently enforced, are dogs, cats, hamsters, rabbits, guinea pigs, nonhuman primates, and marine mammals.

**Antibody:** Proactive proteins produced by lymphocytes (type of white blood cell) that can specifically bind foreign substances.

**Biological Model:** A surrogate or substitute for a process or organ of interest to an investigator. Animals or alternatives can serve as biological models.

**Biological Testing:** The repetitive use of a standard biological test situation or protocol employing different chemicals or different test parameters. Such test protocols are more stereotyped than those used in research, and may be more amenable to the institution of a computerized data retrieval system.

**Biomedical Research:** A branch of research devoted to the understanding of life processes and the application of this knowledge to serve humans and animals. A major user of animals, biomedical research affects human health and the health care industry. It is instrumental in the development of medical products such as drugs and medical devices, and in the development of services such as surgical and diagnostic techniques. Biomedical research covers a broad spectrum of disciplines, such as anatomy, biochemistry, biology, endocrinology, genetics, immunology, nutrition, oncology, and toxicology.

**Blast Overpressure:** The concussion that results when weapons such as artillery pieces are fired. Soldiers firing these weapons can be severely injured by the local pressure effects resulting from weapon use. Blast overpressure occurs when soldiers are fired upon also, i.e., the shock wave from enemy weapon fire/blast.

**Carcinogen:** An agent or process that significantly increases the incidence of abnormal, invasive, or uncontrolled cell growth in a population. Carcinogens fall into three classes: chemicals, viruses, and ionizing radiation. A variety of screening assays have been developed to detect chemical carcinogens, including the *Salmonella*-mediated mutagenesis assay (Ames test), the sister chromatid exchange assay, and traditional laboratory animal toxicity tests.

**Carcinogenesis:** The process by which a change to a cell occurs that leads to cancer.

**Cell Culture:** Growth in the laboratory of cells isolated from multicellular organisms. Each culture is usually of one type. Cell culture may provide a promising alternative to animal experimentation, for example, in the testing of mutagenicity, and may also become a useful adjunct in repeated-dose toxicity testing.

**Chemotactic:** To attract by release of a chemical. For example, cells are attracted to a site of tissue damage by the release of chemicals by the injured cells.

**Computer Simulations:** The use of specially devised computer programs to simulate cells, tissues, fluids, organs, and organ systems for research purposes: to develop mathematical models and algorithms for use in toxicity testing, and to simulate experiments traditionally done with animals for educational purposes.

**Distress:** Usually the production of pain, anxiety, or fear. However, distress can also occur in the absence of pain. For example, an animal struggling in a restraint device may be free from pain, but may be in distress. Distress can be eased with tranquilizers.

**Draize Eye Irritancy Test:** A test that involves placing a single dose of a test substance into one eye of four to six rabbits (the other eye remains untreated) and observing its irritating effects. A promising alternative to this test is the chick embryo chorioallantoic membrane assay.

**Education:** The aspect of education dealt with in this assessment is the use of animals and alternatives in the teaching of life sciences to health professionals and preprofessionals, and research scientists.

**ELISA (Enzyme Linked Immunosorbent Assay):** An assay system that uses antibodies conjugated to enzymes. The amount of antibody attached to the molecule being analyzed can be detected by adding compounds that are cut by the enzyme releasing a colored product which can be quantified.

**Ex vivo:** Outside the living body: denoting removal of an organ, tissue or cells.

**Guidelines for Animal Care and Use:** Various organizations outside the Federal Government have adopted their own guidelines -- e.g., the American Psychological Association's *Guidelines for Ethical Conduct in the Care and Use of Animals*, which is comprehensive and has been endorsed by FASEB; the American Physiological Society's *Guiding Principles in the Care and Use of Animals*; and the American Veterinary Medical Association's *Animal Welfare Guiding Principles*. For federal guidelines, see Interagency Research Animal Committee, NIH *Guide for the Care and Use of Laboratory Animals*, and PHS *Policy*.

**Institute of Laboratory Animal Resources (ILAR):** A component of the National Research Council, ILAR performs periodic surveys on the use of laboratory animals.

**Institutional Animal Care and Use Committee (IACUC):** An institutional committee that reviews research proposals and oversees housing and routine care of animals. The committee's membership generally includes the institution's attending veterinarian, a representative of the institution's administration, users of research animals, and one or more nonscientist and lay member.

**Invertebrate:** Any nonplant organism without a spinal column, e.g., worms, insects, and crusta–

ceans. Invertebrates account for 90 percent of the Earth's nonplant species. For the purposes of this assessment, invertebrates are not considered to be animals.

**In vitro:** Literally, in glass; pertaining to a biological process or reaction taking place in an artificial environment, usually a laboratory. Human and animal cells, tissues, and organs can be cultured *in vitro*. *In vitro* testing may hold some promising alternatives to animal testing, e.g., in testing for eye irritation and mutagenicity.

**In vivo:** Literally, in the living; pertaining to a biological process or reaction taking place in a living cell or organism.

**Macrophage:** A white blood cell that is very active in inflammatory responses and in engulfing foreign objects such as bacteria.

**Mutagenesis:** An agent that induces chemical changes in genetic material. Chemicals, viruses, and ionizing radiation can be mutagenic. Most carcinogens are mutagens; therefore, many screening tests to detect carcinogens are designed to detect the mutagenic potential of the compound. Some mutagens are not direct acting, requiring metabolic activation in the body before they exert their mutagenic potential.

**National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*:** Revised in 1985, the *Guide* details standards for animal care, maintenance, and housing. Its provisions apply to all research supported by NIH, and it is used by many animal research facilities, both within and outside the Federal Government. AAALAC and PHS also use it when assessing research facilities for accreditation.

**Organ Culture:** The attempt to isolate and maintain animal or human organs in *in vitro* culture. Long-term culture of whole organs is not generally feasible, but they can be sustained in cultures for short periods (hours or days).

**Pain:** Discomfort resulting from injury or disease. Pain can also be psychosomatic, the product of emotional stress. Pain can be induced by mechanical, thermal, electrical, or chemical stimuli, and it can be relieved by analgesics or anesthetics.

**Public Health Service *Policy on Humane Care and Use of Laboratory Animals*:** Revised in 1985, the Policy applies to PHS-supported activities involving animals (including those of NIH). It relies on the NIH *Guide for the Care and Use of Laboratory Animals*, and uses institutional committees for the assessment of programs and maintenance of records.

**Polymerase Chain Reaction:** A molecular biological system in which pieces of genetic material can be synthesized in large amounts *in vitro*. This material can be used in diagnostic testing, genetic studies, or for a large number of molecular biological purposes.

**Protocol:** The written plan of a scientific experiment or treatment.

**Reduction:** Considered an alternative to animal use when fewer animals are used in research and education through changed practices, sharing of animals, or better design of experimental protocols.

**Refinement:** An alternative to animal use by better use and modification of existing procedures so that animals are subject to less pain and distress. Examples of such refinements are the administration of anesthetics and tranquilizers, humane destruction, and the use of noninvasive imaging techniques.

**Replacement:** An alternative to animal use, replacing methods using animals with those that do not. Examples include the use of a placenta instead of a whole animal for microsurgical training, the use of cell cultures instead of mice and rats, the use of non-living systems, and the use of computer programs.

**Research Facility:** Under the Animal Welfare Act, any individual, institution, organization, or postsecondary school that uses or intends to use live animals in research, tests, or experiments. Facilities that receive no federal support for experimental work and that either purchase animals only within their own state or that maintain their own breeding colonies are not considered research facilities under the act, however.

**Sporozoite:** The infectious stage of the malarial parasite that is transmitted by mosquitoes.

**Testing:** Standardized procedures that have been demonstrated to predict certain health effects in humans and animals. Testing involves the frequent repetition of well-defined procedures with measurement of standardized biological endpoints. A given test may be used to evaluate many different substances and use many animals. Testing is used to establish the efficacy, safety, and toxicity of substances and procedures.

**Tissue Culture:** The maintenance *in vitro* of isolated pieces of a living organism. The various cell types are still arranged as they were in the original organism and their differential functions are intact.

**Toxicity Testing:** The testing of substances for toxicity in order to establish conditions for their safe use. There are now more than 50,000 chemicals on the market and 500 to 1,000 new ones are introduced each year.

**Vesicant:** A chemical agent that causes burns and tissue destruction both internally and externally.

**Veterinary Medicine:** The science and art of prevention, cure and/or alleviation of disease and injury in animals. Veterinary medicine includes the management of animal care and use programs.

# SECTION VIII
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